The human spleen after trauma

Leemans, Rob

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Saving techniques and autotransplantation

Rob Leemans

Self-portrait of Albert Dürer from 1525.

”Where the yellow spot is and pushing with the finger there, that is the place where it hurts”.

(Bremen, Kunsthalle)

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SAVING TECHNIQUES

AND AUTOTRANSPLANTATION

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Rob Leemans

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Promotores: prof. dr. W. Timens
prof. dr. H.J. Klasen
prof. dr. T.H. The

Promotiecommissie: prof. dr. R. Pabst
prof. dr. M.J.H. Slooff
prof. dr. B.J.M. Zegers

Paranimfen: drs. P. Klinkert
drs. F. Stolte

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Aan Pietsje

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CONTENTS

Chapter 1

General introduction

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Ann Surgery 1992; 215: 256-260.

1. HISTORICAL REVIEW

The spleen has been a mysterious organ for ages, and has often been a subject of study. In ancient times the spleen was thought to be related to the digestive system. Erasistratus believed that the spleen maintained the symmetry of the abdomen, but had no further function. Plato claimed that its function was to keep the liver ”bright and shining”. Hippocrates proposed a vital balance of four essential humours of the body: blood, phlegm, golden bile and black bile. In spite of the lack of anatomical and histologic knowledge in this period, he had described the anatomy of the spleen with remarkable accuracy. The description of function, however, was described different from that of today; the liver was supposed to be the source of golden bile and the spleen of black bile. Galen believed that ”humours unsuitable for its nutriment are discharged by the spleen through a canal into the stomach”. He called the spleen: ”Splenum mysterii organon”[1].
During the 17th and 18th century the main contributions to the study of the spleen consisted of careful anatomical dissections. In 1777 William Hewson recognized associations with the lymphatic system. Rudolph Virchow demonstrated in 1846 that the follicles in the spleen were related to the white blood cells and in 1885 Ponfick recognized the ability of the spleen to remove particles from the blood[2]. About thirty years later, Morris and Bullock described the spleen as an important organ in the resistance to infections[3]. O’Donnell reported a case of ”acute septicemia” in a 6-year-old boy 2 years after splenectomy in 1926. The boy’s father who had had a splenectomy in 1919 also died ”of septic pneumonia, manifesting a similar lack of resistance to the disease”[1]. The role of the spleen in resistance against infections[4] was discussed by Perla and Marmoston in 1935.
It was only after the publication of King and Shumacker on postsplenectomy infections in 1952[5], that there was a rise in concern over the decrease in resistance against infections as a consequence of splenectomy. After this publication the immunological aspects of the spleen became increasingly the targets of scientific interest.

2. THE SPLEEN

Anatomy and histology

Gross

The spleen is a soft, vascular lymphatic organ with roughly the size of a clenched fist and with the shape of a bean. It contains the largest aggregation of lymphoid tissue in the body and it has a central position in the mainstream of the blood vascular system.
The size of the spleen of an adult varies from 12 to 15 cm in length, 4 to 8 cm in width and 3 to 4 cm in thickness. The average weight is about 140 g. in the adult female and about 180 g. in the adult male. It lies in the shelter of the 9th to 11th rib at the left side of the abdominal cavity[6] (*fig. 1*).

The spleen is soft in consistency and friable and is shaped by adjacent, firmer viscera. Together with the visceral peritoneum it forms strong suspensory attachments to the stomach (gastrosplenic ligament), diaphragm (phrenicosplenic ligament), kidney and pancreas (splenorenal or pancreaticosplenic ligament), colon (phrenicocolic ligament) and sometimes with a peritoneal fold to the abdominal wall on the left posterolateral aspect[7].
A long fissure can be seen on the medial side of the spleen; this forms the hilus and is the site of the main entrance and exit for the blood vessels[6].
Around the spleen is a strong fibrous capsule with collagenous trabeculae extending into inside the pulp. The splenic parenchyma can be divided into white and red pulp as can be seen on fresh surgical specimens.

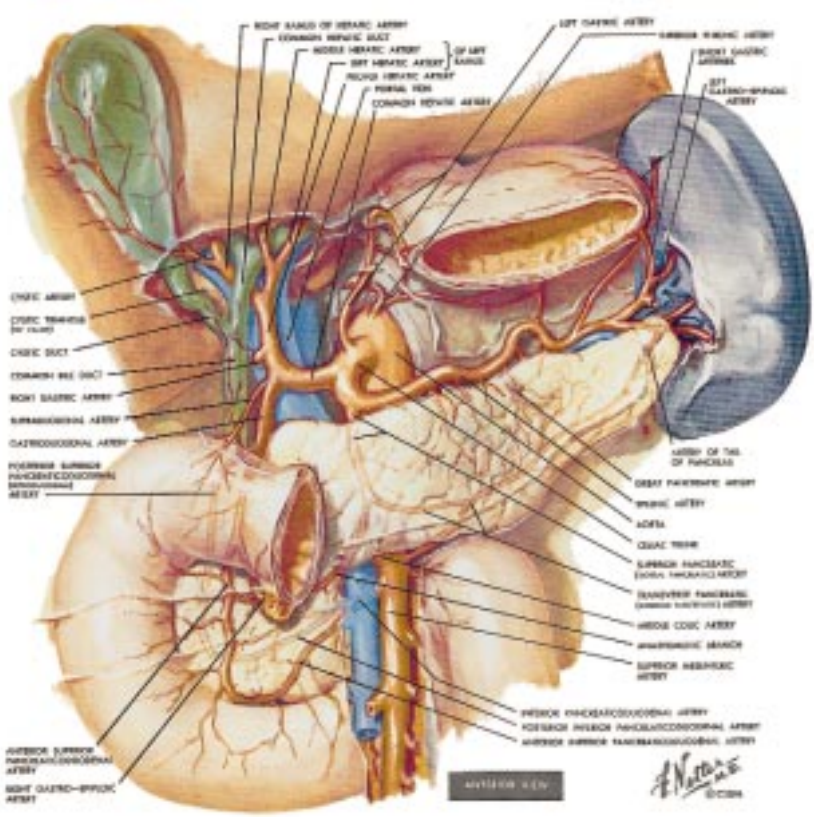


Fig. 1 Anatomy of the upper abdomen with respect to the spleen. (With permission from: Netter, Ciba Collection of medical illustrations, volume 3, Digestive System, New York 1975).

Histology

The spleen consists of two general components: the white pulp (± 5-20%) and the red pulp (± 85%), enclosed by a capsule and interspersed by trabeculae[8].

Capsule

The capsule is composed of dense connective tissue with a few smooth muscle cells. This reflects the minimal contractile capacity of this capsule in man (in dogs it is highly contractile). Serosa covers the capsule except at the hilus where vessels enter the spleen. From the inner surface of the capsule a branching network of trabeculae subdivides the spleen into communicating compartments. These trabeculae carry the blood and lymph vessels into the splenic pulp[9].

White pulp

The white pulp is composed of 3 major compartments that are easily recognized in routinely stained histological sections (*fig. 2*). These compartments are the periarteriolar lymphocyte sheet (PALS), the lymphoid follicle (LF) and the marginal zone (MZ)[10,11].
The PALS is the T-lymphocyte compartment of the white pulp in which T-lymphocytes are interspersed in concentric layers of stromal cells around a central artery. The lymphocytes of the PALS are mostly recirculating cells. The PALS is a site of T-cell clonal expansion. A small percentage of the T-cells within the PALS are in an activated state, demonstrated by IL2-receptor (CD 25) expression. The other T-cells are in a resting state[10,11].

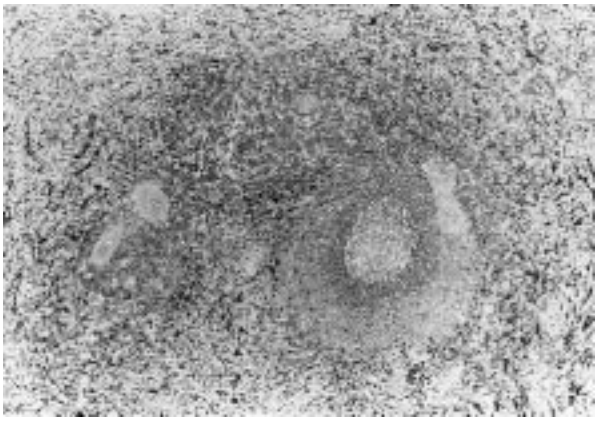


Fig. 2 A: Cross section of the spleen with white and red pulp zones (Giemsa-stained). B: schematic representation of A. RP: red pulp. GC: germinal centre. LC: lymphocyte corona. MZ: marginal zone. P: periarteriolar lymphocyte sheath. A: arteriole (With permission from: Lymphocyte compartments in human spleen, W.Timens, S.Poppema[11]).

The LF of the spleen are globular structures attached to the PALS with similar structure as lymphoid follicles in other lymphoid organs[12]. They can be differentiated into primary and secondary LF. The primary LF consists of a homogeneous aggregate of small B-cells in an inactivated state. Upon activation the primary LF will become a secondary LF with a germinal centre (GC) surrounded by a small rim of remaining small B-cells, the lymphocyte corona (LC). This germinal centre of the secondary LF consists of differentiated B-cells (centroblasts and centrocytes), a few T-cells and follicular dendritic reticulum cells. In the lymphoid follicles a special type of dendritic cells (follicular dendritic cells) is found, which are able to bind immune complexes. They can maintain immune complexes on their cell surface for a long period without phagocytosis. This seems to play a role in the down-regulation of the production of plasma cells[13]. Germinal centres provide a site for rapid proliferation of B-cells with isotype switching and affinity maturation. Upon maturation of the germinal centre, cell division stops and cells differentiate into memory cells or plasma cells, which acquire the ability to migrate out of the germinal centre[14]. A specific type of mononuclear macrophages is present, that phagocytose defective lymphoid cells and debris in the germinal centre[14].
The germinal centre is surrounded by a small border of small lymphocytes (in fact the pre-existent cells of the former primary follicles); this is called the corona or mantle zone. The corona in its turn is enclosed by medium-sized lymphocytes, mostly B-cells, and this is called the marginal zone (MZ). The MZ is an anatomical demarcation between the white pulp and the red pulp. The real border is formed by the perifollicular zone[15]. A great part of the arterial circulation within the MZ terminates intercellularly and at the outer side of the MZ sinuses are present, which are smaller than the sinusoids of the red pulp. The MZ provides an environment, which by its low blood flow allows a prolonged and intimate contact between antigens in the bloodstream and the lymphocytic system[11,14,16]. Lymphoid cells in the MZ have been demonstrated to possess surface immunoglobulin (mainly IgM, in absence of IgD), as well as receptors for Fc-fragments and complement factors (C3b and C3d)[11,14,16]. It is because of this low flow in combination with a specific type of B-cells that the MZ is supposed to have an important role in the primary immune response to T-cell independent antigens type 2 (TI-2 antigens) like the polysaccharide encapsulated bacteria e.g. pneumococci, meningococci and haemophilus influenzae. The marginal zone is a distinct anatomical lymphocyte compartment in the spleen with unique immunohistological features[11].

Red pulp

The red pulp consists of a loose reticular tissue rich in capillaries and venous sinusoids. These sinusoids comprise approximately 30% of the volume of the red pulp. They form a meshwork with many interconnections but also bulb-like extensions with blind ends projected into the cord tissue[15]. The sinusoids have an unique endothelium of longitudinally arranged cells. These run parallel to the long axis of the sinusoids like the staves of a barrel and possess close junctional complexes at regular intervals along their lateral

surfaces to the white pulp veins. Slit-like spaces, which can be penetrated by cells flowing from the pulp cords, separate the endothelial cells. The basal membranes have been shown to contain actin and myosin which can probably contract to vary the tension in the endothelial cell and the dimensions of the interendothelial slits[14]. The interendothelial slits are a critical point in the pathway of particulates through the spleen and in the filtration function. Part of the red pulp tissue has a reticuloendothelial nature with small aggregates of B- and T-lymphocytes and many mononuclear phagocytes. Morphometrically, the size of the lymphoid, non-filtering red pulp compartment seems to equal that of the white pulp[17]. The macrophages are not simply phagocytic cells, but have also secretory capacities and enhance in this way the immunogenicity of antigens. They have the ability to produce components of complement factors, interferon, haematopoetic colony-stimulating factors and fibroblast stimulating factors. This whole system is part of the so-called mononuclear phagocytic system (MPS)[18].

Vasculature and innervation

As 5-10% of the cardiac output at rest passes the spleen, the spleen has to be richly vascularized[9,10]. The splenic artery is the largest of the three branches of the celiac artery, which originates from the abdominal aorta. After passing the upper body of the pancreas horizontally, giving a few branches to the stomach (left gastro-epiploic artery and short gastric artery) and pancreas (large pancreatic artery) the splenic artery divides into several branches about 3,5 cm before the spleen. These branches will divide further, into superior and inferior branches, subdividing into several smaller branches and finally enter the spleen in the hilus. Ramifications of the splenic arterial branches develop internally into trabecular arteries, which pass through the white pulp as central arteries, branches of which supply the lymphatic nodules in the white pulp. From the centre of the lymphatic node the artery can pass through to the red pulp or split into branches in the marginal zone. Via marginal zone sinuses the blood can also reach the red pulp[6].
The red pulp is assumed to have two systems for the blood circulation, which will be described under ”Microcirculation”. The venous drainage commences in the venous sinusoids, located in the red pulp, subsequently draining into trabecular veins. The trabecular veins terminate in branches that unite to form the splenic veins at the hilus of the spleen. The splenic vein passes along the dorsal and superior part of the pancreas and with the superior mesenteric vein becomes the portal vein. At a short distance before the superior mesenteric vein, the inferior mesenteric vein empties into the splenic vein[6,7].
The lymphatic vessels in the spleen are few in number and not as extensively distributed as the blood vessels. Lymphatic capillaries originating in the splenic capsule and trabeculae converge in lymph nodes of the hilus outside the spleen and subsequently pass to lymph nodes along the splenic artery and the celiac axis[6,7].

The splenic nerve supply originates from the celiac plexus. It follows the splenic artery in the hilus and innervates the musculature of the branching vessels. Also preganglionic

parasympathetic fibres of the right vagal nerve follow the splenic artery into the spleen[6].

Microcirculation of the blood

The microcirculation of the blood in the spleen is perhaps the most complex of any organ in the body. It contains blood with a packed cell volume twice that of arterial blood. Most studies of the microcirculation in the spleen have been performed in animals and the results were often extrapolated to the human spleen. It is not clear whether these results are sufficiently representative for the human situation, because the histology and subsequently the micro-anatomy of the human spleen seems to be different from spleens in animals[11,15,19]. However, because of difficulties in investigation of the spleen in man, we have to rely on well designed animal experiments to provide useful hints in the elucidation of the complex mechanisms in the human spleen.
The spleen constitutes the only organ specialised for the filtration of blood. It has been suggested that there is a fast and a slow pathway of the bloodstream in the red pulp of the spleen for which two compartments are assumed to exist for this bloodstream within the spleen. The first system is the closed circulation with direct connection via the sinusoids and collecting veins to the trabecular veins (*fig. 3*). The second (more important) system is the open circulation with arterial vessels ending blindly in the red pulp cord spaces. From the cords the blood runs intercellular and is subsequently collected in sinusoids from which it will be transported by pulp veins to trabecular veins. The fast compartment is intra-vascular, whereas the slow compartment is in the reticular meshwork [17,20].
Some arterial capillaries of the red pulp show cyclic changes in luminal calibre, with sometimes a very low to absent flow. Erythrocytes pass through interendothelial slits in venous sinus walls always from the reticular meshwork into the sinuses[21].

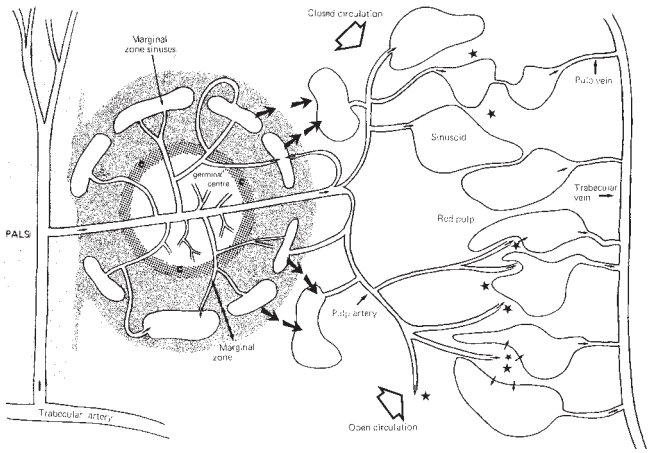


Fig. 3 Schematic cross section of the spleen with blood circulation; c: corona, *: cords of Billroth (With permission from: The Human Spleen, W.Timens[22]).

Lymphocyte circulation

A unique feature of lymphocytes, in contrast to all other cells of the blood, is their continuous migration between lymphoid and non-lymphoid organs through the lymphatic and blood vessels. Granulocytes and monocytes mostly remain in organs once they have left the bloodstream, but lymphocytes may temporarily leave the bloodstream and return to it at later stage (lymphocyte recirculation). This recirculation of lymphocytes is important for the ability to recognise antigens throughout the body and for the interaction between accessory and lymphoid cells in initiating immune reaction[15]. The extent of lymphocyte recirculation in the spleen by the blood far outweighs the total number of lymphocytes using the classical route via lymph vessels and thoracic duct. In a young adult man about $2{,}5x10^{14}$ lymphocytes recirculate through the spleen per day; which is approximately 8 times more than through all lymph nodes[23]. The lymphocytes enter the spleen through the arterial bloodstream and migrate to several splenic compartments. T-lymphocytes rapidly enter the central part of the periarteriolar lymphatic sheaths (PALS), while B-lymphocytes persist in more peripheral parts of the PALS and by 24 hours are evenly distributed throughout the corona. A few migrating B-cells are found in the germinal centres, but no T-cells. It is unknown as yet whether the venous route or the lymphatic route is the most important outflow for lymphocytes of the spleen; probably the venous route is more important than that via lymphatic vessels.
The exact migratory mechanisms and routes of the lymphocyte subsets through all the splenic compartments are very complex and have not yet been clarified[10,14].

Functions of the spleen

The spleen is a unique organ in the immune defence system of the body. It is the only organ which can clear low opsonized antigens from the bloodstream and it is the only organ which is specialised in producing antibodies in a short time after contact with antigens. Besides this, the spleen is a true lymphoid organ with several organised lymphoid compartments.
Because of the central position in the blood stream and the large blood supply of about 5 per cent of the blood volume per minute, the spleen represents an important meeting point between antigenic information transported by the blood and the immune system. It possesses a wide range of the immune cell repertoire and its specific architecture allows unique functions. Two major critical functions of the spleen can be recognized: it serves as a large phagocytic filter and it is a major antibody producing organ.

Filtration

Filtration of the blood is the best known and a (quantitatively) important function of the spleen. The reticular meshwork in the red pulp with the terminal arterial vessels and the venous sinusoid are specialised for filtration of the blood. When blood passes the

endothelial wall of the sinusoids, bloodcells have to pass through the interendothelial slits (*fig. 4*). These slits are only small in diameter, hence during this passage the blood cells have to deform, subsequently to regain their normal form. If the cells lose their deformation capacity or if the cell walls are too fragile, the cells cannot pass through this filtration system. Erythrocytes with intracellular inclusions (pittings, Howell-Jolly bodies, intra-cellular organisms as malaria, etc.) can be cleared of these inclusions during the passage without destroying the entire cell. The membrane of the cells reseals and the cells pass into the sinuses and the general circulation. The perisinusoidal phagocytic cells will clear the inclusions and the aged bloodcells[18,24].

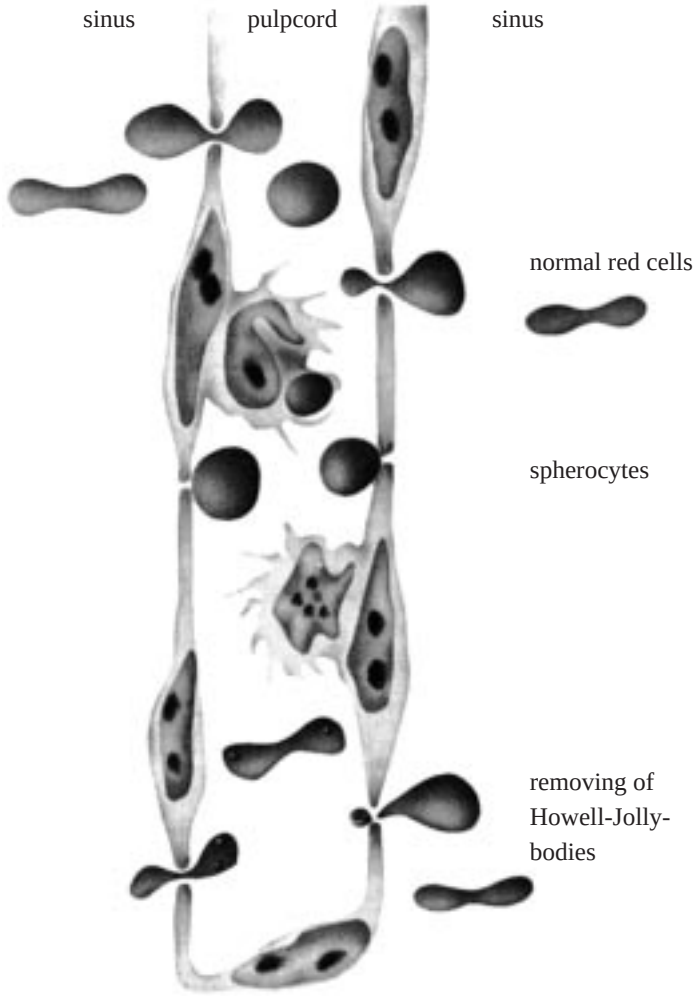


Fig. 4 Passage of an erythrocyte to a sinusoid (With permission from: Immuno-architecture of regenerated splenic and lymph node transplants, R.Pabst, J.Westermann, H.J.Rothkötter[24]).

Immune function of the spleen[16]

Phagocytosis of foreign particles can be promoted by interaction with opsonins, serum factors which enhance their uptake by specific phagocytosing cells. The spleen has a prominent role in the generation of opsonins[2,25]. Splenic phagocytes, together with macrophages in the liver, synthesise the majority of components of the classical pathway of complement[25]. Generation of specific antibody is primarily dependent upon the spleen. The role of the spleen in phagocytosis of foreign particles is particularly important for non- or badly opsonized particles. Whereas the mononuclear phagocyte system (MPS) in the liver is the main site of phagocytosis of opsonized particles, the spleen is the major organ for the phagocytosis of non-opsonized particles. In experiments in rabbits, the phagocytosing capacity for non-opsonized particles appeared to be sixty times as effective in spleen as in liver when corrected for weight (reviewed by Lockwood)[25]. The unique microvasculature of the spleen supposedly contributes to the specialised function of the spleen in the phagocytosis of insufficiently opsonized particles[9,13,26]. The greatly retarded blood flow in the red pulp cords allows a very intimate and prolonged contact between antigens and phagocytes. Thus, particles can be ingested without specific ligand-receptor interactions. An important practical implication of this specialised phagocytosing capacity is that the spleen is the most important site of clearance in the early phase of bacterial invasion before sufficient amounts of specific antibody have been produced. This is particularly important for blood-borne, T-cell independent type 2 antigens like polysaccharide encapsulated micro-organisms (e.g. pneumococci)[27].

Another special feature of the spleen is the generation of tuftsin, originating from the Fc-fragment of IgG. This is a tetrapeptide reported to exert stimulatory effects on activity and migration responses of phagocytic cells[28].

The spleen also plays a part in the alternative complement pathway. Complement factors work synergistically with antibodies in promoting phagocytosis of bacteria. In the presence of the complement factor C3b the immunoglobulin (Ig) opsonization degree required for phagocytosis is decreased 100-fold. Moreover, lysis of bacteria can take place by complement factors only too[14]. In the primary immune response to TI-2 antigens C3d is also an important factor and a high density of C3d-receptors is found in the marginal zone of the spleen[29].

The spleen is the site of IgM specific antibody generation very early after exposure to blood-borne antigen. The first contact of antigens entering the spleen via the blood and immunocompetent cells occurs in the marginal zone, a structure exclusively present in the spleen[11,31,32]. This marginal zone is unique in its microvasculature, enabling a very low blood flow, in the presence of specialised macrophages antigen presenting cells and a subset of intermediate-sized B-cells with a specific phenotype: IgM+, IgD-, and strongly CD21+[11,26,31,32]. Although B-cells with this phenotype can be found in other lymphoid tissues, the splenic marginal zone contains the largest accumulation of this type of B-cells in the body. When the blood enters the marginal zone sinusoids, there is a considerable increase in flow area diameter, with a subsequent decrease in blood flow. Similar as in the

red pulp a sluggish flow results; this enables a close contact between antigens and phagocytes or lymphoid cells, and between different cell subtypes involved in the immune response[13,26].

Because the spleen is a major lymphoid organ it also plays an important role in the primary humoral and secondary immune response. After encountering antigens in the MZ-sinuses, antigen-specific B-cells migrate to the lymphoid follicles[14]. From here they can either differentiate to produce antibodies (plasmacells) or to B-memory cells. The primary immune response is very important and can provide antibody production within 6 hours after the first contact with an antigen. The MZ B-cells are particularly well equipped for rapid and easy activation in a primary immune response[32].

The spleen is also of importance in the secondary immune response, because the formation of memory cells of B- and T-lymphocytes is especially promoted by the spleen[22,25,27].

The spleen is specifically involved in the immune response to thymus-independent antigens type 2 (TI-2 antigens). These antigens, generally polysaccharides, are the antigenic component of the capsule of encapsulated bacteria such as Streptococcus pneumoniae, Haemophilus influenzae and Neisseria meningitides. The immune response to TI-2 antigens is characterised by the need of T-cell produced factors, although it is independent of the actual presence of T-cells[33]. After splenectomy this response is significantly decreased or even absent. The initiation of the response to polysaccharide antigens (TI-2 antigens) takes place in the splenic marginal zone. In rodents, TI-2 antigens were found to localise specifically on antigen-presenting cells in the marginal zone and the elimination of MZ cells abrogated the immune response to such antigens[35]. As described above, marginal zone B-cells have a distinct immunophenotype. By their high expression of CD21, the receptor for complement fragment C3d, MZ B-cells play a specific role in the immune response to TI-2 antigens, as these antigens are able to bind C3d[26,29]. In this way, TI-2 antigen-C3d complexes can bind to - and activate marginal zone B-cells[26,29].

Other functions

The spleen has a reservoir function for a large number of all kinds of blood cells by means of a process that is not yet understood. This storage function is mainly for thrombocytes, but also for erythrocytes and lymphocytes. Of all thrombocytes in the body about 30% may be stored in the spleen. The spleen is the largest lymphoid organ with 25% of all white blood cells of the body, mainly lymphocytes. Only 5% of the red cells are supposed to be stored in the spleen.

The number of blood cells in the spleen at a given time depends on the presence or absence of pathology in the spleen and/or of the blood cells. In the reticular meshwork the haematocrit of the blood is twice that of arterial blood. The spleen appears to function as a ”nursery” for reticulocytes after their release from the bone marrow[21], and is supposed to play a role in final maturation. Reticulocytes have a reduced negative surface charge, are less flexible, contain unneeded organelles and are bigger than mature red cells. The reticulocytes will be sequestered in the red pulp for two days because of these properties

thus allowing them to mature. After maturation the erythrocytes will be remodelled and then emerge into the circulation[27].
Additional known functions of the spleen are haematopoiesis during fetal life, a positive effect on factor VIII serum levels, inhibition of serum angiotensin-converting enzyme activity, and participation in reutilization of iron from destroyed erythrocytes[27,36].
It is even suggested that the spleen has a role in lipid metabolism, with lower HDL-cholesterol and higher triglyceride serum levels after splenectomy[37].
A summary of the functions of the spleen is given in table I.

Table I Functions of the spleen[16,27,38].

White Pulp	Red Pulp
- Antibody synthesis - Initiation of humoral response to -TI2 antigens badly opsonized particles) - Reservoir of lymphocytes	- Filter function - Phagocytosis (in particular - Reservoir of thrombocytes and immature erythrocytes - Haematopoiesis (fetal life) - Tuftsin production - Role in alternative complement pathway - Positive effect on factor VIII - Reutilization of iron - Inhibition of angiotensin-converting enzyme

3. CONSEQUENCES OF SPLENECTOMY

Although incidental reports mentioned a relationship between splenectomy and infection, it was not until 1952 that a causative association was reported between splenectomy (for congenital haemolytic anaemia) and the occurrence of meningitis with sepsis[5]. Since then the increased risk of infection and septicemia directly related to splenectomy has been well defined in the literature. Such infections are now generally termed ”Overwhelming Post Splenectomy Infections” (OPSI).
In most cases the OPSI syndrome is caused by one of the following micro-organisms: Streptococcus pneumoniae (50%), Neisseria meningitides (12%), Escherichia coli (11%), Haemophilus influenzae (8%) and Staphylococcus aureus (8%), but also by mycobacteria, viruses and parasites[39,40,41].
The frequency of OPSI is dependent on age and the cause of splenectomy. The highest frequencies were found after thalassaemia and Hodgkin’s disease and the lowest

frequencies after trauma[42]. Singer came to an overall frequency of 4.25% with a mortality of 2.52%. In patients who had had a splenectomy for traumatic splenic rupture the mortality due to sepsis was 0.58%, after thalassaemia however it was 11.0%. In the total population the incidence of mortality due to sepsis was 0.01%[43]. In a more recent review about OPSI in 12514 postsplenectomy patients (with 5902 sufficient reports), under 16 years of age an OPSI frequency of 4.4% was found with a mortality of 2.2%, but for adults these figures were 0.9% and 0.8% respectively. Overall there was 3.6% morbidity and 1.8% mortality.
The highest incidence of OPSI is generally found in infancy and childhood. Patients who have undergone splenectomy for haematologic diseases, reticuloendothelial diseases or portal hypertension have a higher incidence than those undergoing splenectomy for trauma[39,43,44,45,46,47].
Most frustrating is the high overall mortality rate of OPSI of about 50%[9,10,27,36,39,40,42,48,49]. As indicated above after-splenectomy there is a significant decrease in the primary immune response to bacterial capsular polysaccharide antigens[39,40,41]. These antigens belong to the group of TI-2 antigens, and other antigens of this type also give a similar decreased immune response after splenectomy[50,51]. Another cause for the increased risk of OPSI is a decrease in phagocytic activity, in particular with respect to phagocytosis of poorly- or non-opsonized antigens. After a splenectomy the phagocytic function will be partly taken over by the liver. However, the liver needs a higher level of antigen opsonization. This may present an important problem especially with respect to thymus independent type 2 antigens like encapsulated bacteria which are badly opsonized[50], in particular because also the spleen dependent specific TI-2 antibody response is hampered. A lower phagocytic activity also results from decreased tuftsin concentrations after splenectomy[48,52].
The general ability to generate a specific antibody response after the first contact with a blood born antigen, the primary immune response, is also reduced. This is consistent with a low production of IgM after splenectomy.
The alternative complement pathway also seems to be reduced after splenectomy, with normal functioning of the classical pathway.

After splenectomy the ability of the body to filter the blood will be reduced which results in an increase of erythrocytes with inclusions like vacuoles and Howell-Jolly bodies, and with surface pits. The ability to remove intracellular organisms such as malaria and bartonella is also reduced. The loss of splenic maturation for reticulocytes causes a high percentage of immature erythrocytes and reticulocytes in the bloodstream[27].

Another, less important impairment that can have consequences is a decreased reservoir function for blood cells. There will be an increase of thrombocytes and a prolonged residence time of lymphocytes in the blood shortly after splenectomy. However, after a few months the thrombocytosis seems to be reduced to normal[27].
The effects of splenectomy in humans and animals are summarised in table II.

Table II Effects of splenectomy

Immunological	Non-immunological
- Reduced phagocytic activity of badly opsonized antigens - Decreased tuftsin formation - Lower IgM serum level - Prolonged residence time of lymphocytes in blood - Reduced alternative complement pathway activity - Increased auto-antibody activity - Diminished numbers T-suppressor cells	- Reduced filter function - Increase of reticulocytes - Increase of platelets

4. PRESERVATION OF SPLENIC FUNCTIONS

Spleen salvage techniques

From the time the OPSI syndrome was recognized to be related to splenectomy, it still took several years before attempts were made to diminish the risk of infection. One approach has been the introduction of spleen-saving techniques. Several techniques have been described concerning the management of the various degrees of splenic rupture. For an evaluation of this techniques Shackford et al. published a grading system which was modified for clinical use[53,54,55]. Later on the grading of the American Association for the Surgery of Trauma (AAST) came in use[56] (*table III*).

There is a wide variety of techniques aimed at splenic preservation[57], being summarised in table IV and figure 5.
First of all the non-operative treatment with close observation should be considered. This is only possible in haemodynamically stable patients who are conscious and without associated abdominal injury. Mostly these patients are kept on the intensive care with frequent checking the circulatory parameters. Blood samples are taken regularly for evaluation of haemoglobin and haematocrit and sonography or CT-scan of the abdomen should be performed. This regime can be applied quite safely in patients with a capsular tear (grade I or II rupture). Traub, Wiig and Pearl et al. described good results of this therapy[58,59,60]. Cogbill et al. published a multi-centre study involving 112 splenic injuries, treated by nonoperative management[61]. In 13 cases a laparotomy was needed (5 splenectomies and 8 splenic salvage procedures) without mortality. Based on this experience they extended their criteria for selective nonoperative management of blunt splenic injuries even to class III.

Table III. Modified grading system of splenic ruptures according to Shackford[53,54,55] and the American Association for the Surgery of Trauma [56].

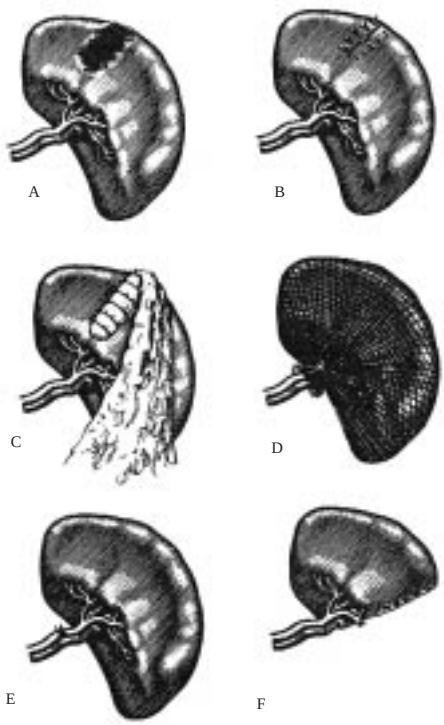
GRADE:	SHACKFORD	AAST
I	Localised capsular rupture without significant parenchymal injury.	Haematoma subcapsular < 10% surface. Laceration capsular < 1 cm deep.
II	Localised capsular rupture with local parenchymal injury.	Haematoma subcapsular 10-50% surface or parenchymal < 5 cm diameter. Laceration parenchymal 1-3 cm deep.
III	Parenchymal injury not extending into the hilus or involving major vessels.	Haematoma subcapsular >50% or parenchymal > 5cm. Laceration parenchymal > 3cm or involving trabecular vessels.
IV	Severe parenchymal injury extending into the hilus or involving major vessels.	Laceration involving segmental or hilar vessels.
V	Completely shattered or fragmented spleen or separation from the blood supply.	Completely shattered spleen or devascularisation.

Table IV. Therapeutic strategies in the management of ruptured spleen.

- non-operative: observation.
- haemostatic agents: thrombin, gelatine foam, collagen cyanoacrylate adhesive.
- arterial ligation: main trunk, segmental vessels.
- splenorrhaphy: mattress sutures, omental wrap, absorbable net.
- partial splenectomy: stapling, laser, sutures.
- total splenectomy: autotransplantation, (splenosis).

Later on the nonoperative management of splenic injury has been described with a high success rate[62,63] even in patients with other (neurologic) injuries[64,65]. In the last 5 years the nonoperative management of splenic injury has become more accepted and got a place in the techniques of treatment[56,66,67,68]. At present this approach is still a matter of debate because of the risk of delayed complications of nonoperative management of the splenic injuries[69].

Fig. 5 Schematic drawings of spleen salvage techniques. A: haemostatic agent. B: mattress sutures. C: omentum application. D: absorbable net. E: ligation of artery. F: partial splenectomy (With permission from: Splenectomy, M.J.Cooper, R.C.N.Williamson[57].



For the operative treatment of spleen rupture various techniques can be used[55,57] alone or in combination (*see table IV page 25*).

The use of haemostatic agents is one of the possibilities for treatment of splenic injuries. Coln et al. described a trial with Gelfoam®, Avitene®, Surgicel® and Collastat® in rabbits and the best results were archived with the use of Collastat® [70] . In 1990 Krar et al. and Ochsner et al. used fibrin glue in patients with splenic and hepatic injuries, also with good results[71,72]. Because of the risk of clot displacement this therapy seems to be suitable only

for minor injuries of the spleen or in combination with other techniques such as splenorraphy[73]. It is suggested that there may be a place for fibrin glue in the laparoscopic approach of abdominal trauma[74]. Although in a number of publications the laparoscopic approach was considered as valuable, this technique is still under discussion[75,76,77].
Successful use of an argon beam coagulator in splenic injury has been described in an animal experiment with pigs, this technique being more effective in treating splenic injuries than the use of other conventional surgical techniques such as sutures, electro cautery, digital pressure and application of hemostatic agents[78]. This technique seems favourable when combined with laparoscopy.
Clamping the splenic arteries during the operation may provide a temporary arrest of bleeding. Ligation of the splenic artery is feasible in arterial bleeding, when this is not amenable to direct suture. Because the spleen is also nourished by the short gastric and left gastro-epiploic arteries, total necrosis of the spleen will be prevented[79]. Ligation of the main artery is feasible and may permit splenic conservation, but because of reduction of the blood flow through the spleen the phagocytosis of badly opsonized particles will be diminished[80]. This may lead to an increased risk of OPSI. Division of the main artery into several branches occurs outside the spleen and usually only the affected branches need to be ligated. Arterial embolisation has also been described as a technique to treat splenic rupture[81].
Suturing of the spleen can be performed by mattress sutures with or without an omental flap or haemostatic material[57]. To avoid the danger of tearing, Teflon^{R} or other patches can be helpful. This technique is most satisfactory in children because they have a strong capsule, but can also be performed in adults[57,82,83]. However, it remains a risky form of treatment, because of the fragility of the splenic tissue.

An elegant treatment of splenic injuries is that of splenorrhaphy by wrapping the spleen in an absorbable net. It is safe and can be performed together with the use of haemostatic agents[84,85].
Although good results have been described by several authors[86,87,88,89,90,91]. drawbacks do exist[92]. These aspects will be further described and discussed in chapter 2 of this thesis.
In selected cases it is possible to perform a partial splenectomy, for example in case of lesions in the lower pole of the spleen. This technique has been described with good results (also in own experience). Partial splenectomy is enabled by the segmental blood supply of the spleen. Most individuals have two primary lobar intrasplenic arterial branches, so upper or lower pole resection can be performed by the finger-fracture technique or with the use of a laser. Haemostasis of the section can be carried out by mattress sutures, stapling and CO_2 laser. Local haemostasis can also be obtained after ligation of upper- or lower pole arteries[55,61,93,94,95].
Whenever the above-described techniques fail to stop the bleeding of the ruptured spleen or when the rupture is too serious (grade V) a splenectomy has to be performed. This may be combined with autotransplantation of spleen tissue, as will be discussed later. If there are accessory spleens it is advisable to leave them in situ because it is very well possible

that accessory spleens can compensate for some of the impaired functions after splenectomy (discussed in chapter 6 of this thesis).
Several analyses have been reported about the decision processes when facing a ruptured spleen, resulting in decision algorithms[96,97,98].

Autotransplantation after splenectomy

Splenosis peritonei is the outgrowth of small splenic particles everywhere in the peritoneal cavity due to dispersion of spleen particles in traumatic or iatrogenic rupture of the spleen. Griffini and Tizzoni described as early as 1883 areas of spontaneous splenic regeneration in the peritoneum of dogs that had undergone splenectomy[99]. A few years later it was also described in man, but incorrectly called accessory spleens[100]. Kuttner and Faltin considered this phenomenon to be the result of seeding of particles of the ruptured spleen[101]. This hypothesis was proven by Von Stubenrauch and Kreuter who deliberately sowed splenic pulp in the peritoneal cavity, resulting in a large number of small spleen implants which ultimately grew larger than the original particles[100,102].
The condition of splenosis peritonei may have been the stimulus to study the possibilities and therapeutic benefits autotransplantation of splenic tissue. The expression ”splenic autotransplantation” in this thesis represents the transplantation of a part (or all) of someone’s own spleen to a site in the body without formal direct connections to the vascular system, in this way distinct from the technique of vascularised autotransplantation[103,104,105].
”Accessory spleen” means the presence of a congenital extra spleen somewhere in the peritoneal cavity. The frequency of this condition is thought to be about 18%[106].
The term ”splenosis” was first suggested by Buchbinder and Kiphoff in 1939, to describe areas of spontaneous splenic regeneration in the peritoneum after splenectomy for trauma[107].
Pearson et al. reported a reduced percentage of ”pitted” red cells in 13 of 22 children after splenectomy for trauma, suggesting a return of splenic function by splenosis[108]. Nielsen described a positive correlation between a low percentage of vacuolated erythrocytes and the presence of ectopic splenic tissue detected by Tc-scanning[109].
Histological and immunohistochemical studies of splenosis suggested a normal structure of splenic tissue, nearly indistinguishable from normal splenic tissue[110]. It was even advised that spleen tissue resulting from splenosis should not be removed without a specific indication[111].
This splenosis could explain the lower incidence of OPSI after splenectomy for trauma when compared with splenectomy for other reasons[108]. This led to the hypothesis that an autotransplantation at the time of splenectomy might restore at least part of splenic immune function. Experiments in this field were started already by Marine and Manley in 1920. Further studies were performed in animals, e.g. rats, mice, dogs and pigs and later in men to evaluate whether or not autotransplantation might provide (some) protection against OPSI[113,114,115].
Studies were also performed with respect to the ideal site for transplantation (peritoneum, omentum, subcutis) and the quantity of splenic tissue that was needed (a few grams up to

a complete spleen) to reach optimal results[113,114].
Histological studies in rats have shown that regeneration of autotransplanted splenic tissue occurs in phases. First necrosis of the autotransplant will occur. Within a few hours after transplantation the fragments become necrotic, except for a small rim of reticular cells underneath the capsule. All lymphocytes in the transplant die, and only remnants of reticular cells and erythrocytes remains. In the course of the following days, capillaries and reticular fibres grow out to form a subcapsular vascular space. After a week the regenerating tissue differentiates into an outer and an inner zone, with reticulum cells and sinus like spaces in the outer zone. Lymphocyte immigration starts and the red pulp is formed about two weeks after transplantation. In the following weeks the typical white pulp

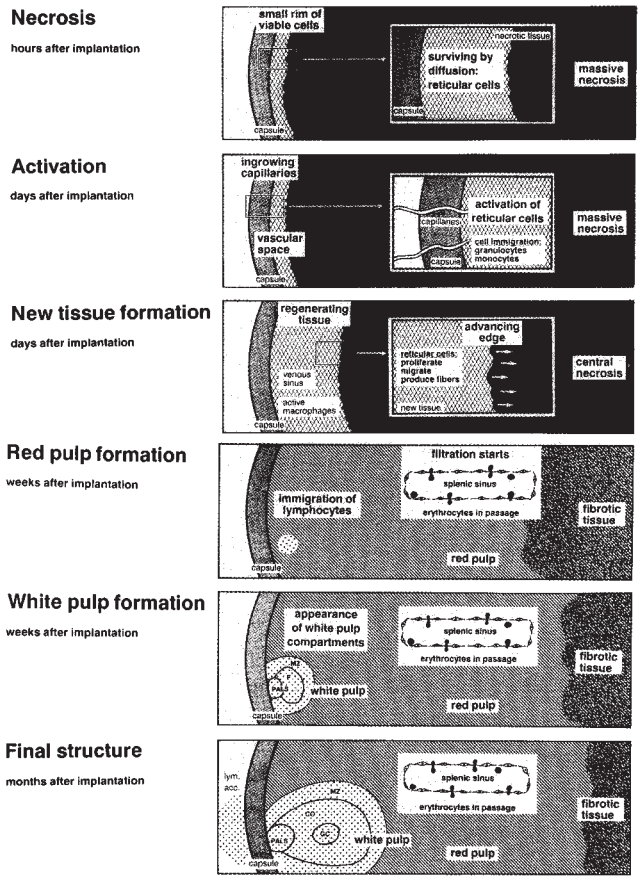


Fig. 6 Schematic drawing of autotransplant regeneration with approximate time points. CO: corona; F: follicle; GC: germinal centre; lym.acc.: lymphocyte accumulation; MZ: marginal zone; PALS: periarteriolar lymphatic sheath. (With permission from: Immunoarchitecture of regenerated splenic and lymph node transplants, R.Pabst, J.Westermann, H.J.Rothkötter[24])

compartments appear in approximately the same order as during ontogeny. First the periarteriolar lymphocyte sheaths (PALS) are formed, followed by the follicles and the marginal zones.

About 3-6 months after implantation the autotransplants have attained their final structure with germinal centres, suggesting a functional capacity of the white pulp[24] (*see also fig. 6*). The histology of the autotransplants after regeneration resembles that of normal spleens but this is misleading in that the relative size of the splenic compartments is different from that of the normal spleen. The PALS and the marginal zone are relatively reduced to 50% of that in the normal spleen and areas of fibrotic tissue do occur, mainly in the central parts of the transplants[24,116,117,118,119].

Although splenic tissue possesses the possibility of regeneration, it is not known which factors regulate this regeneration[120,121]. Orthotopic spleen seems to have a suppressive effect on regeneration of autotransplants, but the suppressive or stimulatory factors are unknown[24,113,118,122]. Pabst suggested a stimulating factor by increased workload to the splenic compartments[123].

The site of transplantation also seems to affect the results of the transplantation. Splenic fragments have been implanted in many different sites, e.g. subcutaneous, intramuscular, subperitoneal, mesentery, small intestines, kidney, and portal vein, but most often in a pouch in the omentum. The best site for implantation seems to be intraperitoneal, especially in the omentum[124,125,126] because of the extensive vascular supply. Although some authors suggested implantation of at least 20-30 cm^3 of splenic tissue[127] the mass of the implanted tissue seems not to influence the result. The technique of transplantation is important. The best result is obtained after implantation of slices or cubes of spleen tissue with fragments of capsule and cords. In this way the chance of survival of the white pulp is the highest[24,118,128].

The age of the patient may influence splenic regeneration: the regenerative capacity and capacity of ingrowing vessels can be expected to be better in young patients than at older age[129,130].

To test the function of autotransplanted splenic tissue and to evaluate whether or not autotransplantation is useful, several studies have been performed, mostly in animals. There are however histological and functional differences between the spleen of animals and man. It is not possible to perform the same tests in humans as in animals[19,38]. Besides that, many different animal species, different autotransplantation techniques and different sites of transplantation have been used. This makes comparison and extrapolation to the human situation difficult. Some conclusions can be drawn[113]. Because the blood flow in the autotransplants was mostly no more than 10% of normal splenic blood flow, a reduced clearance function is to be expected[118]. Horton[80] and Pabst[131] already demonstrated the importance of the splenic blood flow in clearing organisms out of the blood stream[80]. In rats, rabbits and dogs the blood clearance was significantly reduced[132,133,134]. In man a reduction of circulating Howell-Jolly bodies was found after autotransplantation[135,136]. In several studies resistance to induced pneumococcal sepsis in animals after splenectomy was evaluated by injecting bacteria intravenously. The results showed a better resistance in animals with autotransplantation than in those without[133,137,138].

Other positive aspects of autotransplantation have also been described e.g. improved alveolar macrophage function[139], improved phagocyte function in peripheral blood[140], correction of IgM levels[141] and increased pneumococcal antibody titres after vaccination[142]. The effect of autotransplantation on changes in lymphocyte subsets, immunoglobulin levels and complement levels is still a subject of discussion[24,113]. On the other hand: in spite of the presence of some splenic tissue, a number of fatal cases because of OPSI have been published[113,143,144]. Also complications of autotransplantations and of splenosis after splenectomy have been described, such as haemorrhage, abscess and ileus[120,145,146,147,148,149].

Despite all the studies performed so far it is still not completely clear whether and to what extent autotransplantation can give protection against OPSI, although positive arguments have been found.
As appears, reports on the effects of auto-transplantation of spleen fragments are controversial[46,150]. Although beneficial effects have been reported[128,135,151], several other studies observed no significant differences compared to splenectomized patients without splenic regrowth[129,143,144]. Several factors may account for this. First, the total amount of blood that is filtered is low, despite an acceptable vascularisation. Second, the micro-anatomy of the splenic fragments is probably not suited for the specific local low flow that is characteristic for the normal spleen and is essential for the close contact between antigen, and phagocytes and immune responsive cells. Third, for testing of the immune function of the autotransplanted spleen fragments two items have to be evaluated: phago-cytosing capacity, with special attention to non- or badly opsonized antigens; and (humoral) immune response capability, with particular attention to TI-2 polysaccharide antigens. With respect to these items the presently used tests of the function of the auto-transplanted spleen fragments may not be adequate for evaluation of the ability of the fragments to perform real ”splenic” immune functions.

5. AIM OF THIS STUDY

The spleen is an important organ of the immune system and splenectomy will have a negative effect on the immune functions, especially on the primary immune response to bacterial capsular polysaccharide (TI-2) antigens. The question is whether, and in what way, these functions can be preserved after splenic trauma,often followed by splenectomy. The first attempt to preserve splenic function should be to maintain the spleen itself with its own vasculature. In chapter 2 a study is described presenting the results of the use of a new splenic salvage technique with an absorbable net of Vicryl® .
If splenectomy is inevitable, autologous transplantation of parts of the ruptured spleen into the greater omentum is another option to consider to preserve (at least part of) the immunological function of the spleen. Despite the studies performed, there has been a lot of controversy about the benefits of autotransplantation, especially in man[152,153].
In 1984 we started with autotransplantation of splenic tissue after splenectomy for severe

traumatic rupture of the spleen in cases in which the spleen could not be saved. Along with this procedure, a study was started to evaluate whether or not an autotransplantation of splenic tissue in the omentum would have a positive effect on the immunological defence after splenectomy. Splenectomized patients that underwent a spleen autotransplantation were compared with splenectomized patients that did not undergo this procedure.
As in many studies attention has been focussed on the filter and phagocytosis function of the autotransplanted spleen, several tests of these functional capacities were performed. In chapter 3 a study determining the selective splenic Fc-receptor function is described, as a test of the mononuclear phagocyte system capacity of the autotransplants.
The general immune response capacity and the phagocytic activity of patients with and without autotransplants after splenectomy are described in chapter 4. With respect to immunological defence against postsplenectomy (bacterial) infections, special attention was paid to the capability of autotransplanted spleen tissue to mount a specific humoral immune response. An adequate humoral response would enable other non-splenic parts of the mononuclear phagocyte system to clear the opsonized pathogenic bacteria. In chapter 4 we have also included a test of the specific humoral response capacity of the above-described patient groups against pneumococcal polysaccharides, as present in the Pneumovax vaccine.
Consequent on the study involving human subjects, a similar prospective auto-transplantation study has been performed in rats with tests of the primary humoral immune response against different pneumococcal polysaccharides, combined with evaluation of the immuno-architecture of the autotransplanted splenic fragments. The results are reported and discussed in chapter 5.
After accidental splenectomy, accessory spleens may function as a spare spleen, maintaining some of the immune functions. A basic condition to be able to perform adequate splenic immune functions is that the basic architecture of accessory spleens is similar to that of a normal spleen, including spleen-specific lymphoid compartments, like the marginal zone. A morphological and immunohistological study comparing human accessory spleens with their normal counterparts is described in chapter 6.
In chapter 7 the findings described in this thesis are summarized and discussed, including final conclusions. Based on the main findings, a perspective is given, with suggestions for further research.

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Chapter 2

A SPLEEN-SAVING METHOD FOR SPLENIC RUPTURE WITH AN ABSORBABLE NET

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Summary

The treatment of splenic injury has been the subject of extensive discussions. In the past, splenectomy was considered to be the treatment of choice, until it became clear that this caused an increased risk of the overwhelming post-splenectomy infection (OPSI) syndrome. In animal experiments and subsequent clinical reports, it has been described that some ruptured spleens can be saved by wrapping in a resorbable net.

Thirty-one patients with severe traumatic rupture of the spleen were treated by a spleen salvaging procedure to prevent a possible overwhelming postsplenectomy infection (OPSI). A description is given of the technique of wrapping an absorbable net around the spleen. Haemoglobin, haematocrit, leukocyte and thrombocyte values were determined postoperatively. A liver and spleen scan was performed in 7 patients one month post-operatively in order to evaluate splenic function. 30 of the 31 patients showed no sign of bleeding. One patient died as a result of serious brain injury. One patient underwent a re-laparotomy because of a mechanical ileus caused by an adhesion after which a wound infection occurred. In one case the patient received 2 nets because of the extensive rupture of the spleen (grade IV-V), which resulted in a non-septic necrotic spleen. Perisplenic effusion was found in 2 patients, which resorbed spontaneously. No infections occurred in the area of the spleens or laparotomy wound. Altogether there were only 2 serious spleen-related complications (ileus and necrotic spleen) in 30 patients (6.7%).

On the basis of these results, we propose that a spleen-saving method should always be considered as a first option in the treatment of a traumatic rupture of the spleen. The resorbable net has proven to be a major gain in this treatment. Our conclusion is that the use of the absorbable net is a safe but demanding operation technique, which requires experience in the treatment of splenic injury.

Introduction

The treatment of splenic injury has been the subject of considerable debate during the past decades[1,2,3,4]. In the past, splenectomy was considered to be the treatment of choice. However, the removal of the spleen is currently considered to be responsible for the increased risk of the overwhelming post-splenectomy infection (OPSI) syndrome[5]. In addition, insight has been achieved into the physiology of the spleen as well as the role of this organ in immunological defence[6,7].

At present, spleen-saving techniques are preferred to splenectomy in the treatment of splenic rupture. It appears from animal experiments that it is frequently possible to save a ruptured spleen by wrapping it in a resorbable net[8]. Our experience with this treatment modality is presented here.

Patients and methods

In the period from February 1987 until and including December 1989 31 patients with a traumatic splenic rupture were treated with application of a resorbable net. This procedure was carried out in 6 females and 25 males with an average age of 27 (7-69) years. Twenty-eight had had a blunt abdominal trauma, 1 had a penetrating abdominal injury and in one case the injury to the spleen was iatrogenic. The grading system according to Shackford[9] was used in grading the injuries, with a modification according to Kramer (*table I*)[8]. Haemoglobin, haematocrit, leukocyte and thrombocyte values were determined postoperatively. A liver and spleen scan was obtained in 7 patients one month postoperatively in order to evaluate splenic function.

The resorbable net is made from polylactin 910 (vicryl), a copolymer of glycolide and lactide. The diameter of the net is 30 cm. (*fig. 1*) and it is completely resorbed within 3 months. The tensile strength of the net is reduced to 50 % after 15 days (*fig. 2*). The net is wrapped around the injured spleen under some tension using previously attached threads. If necessary the injured spleen is mobilised. In this manner, peroperative haemostasis is achieved.

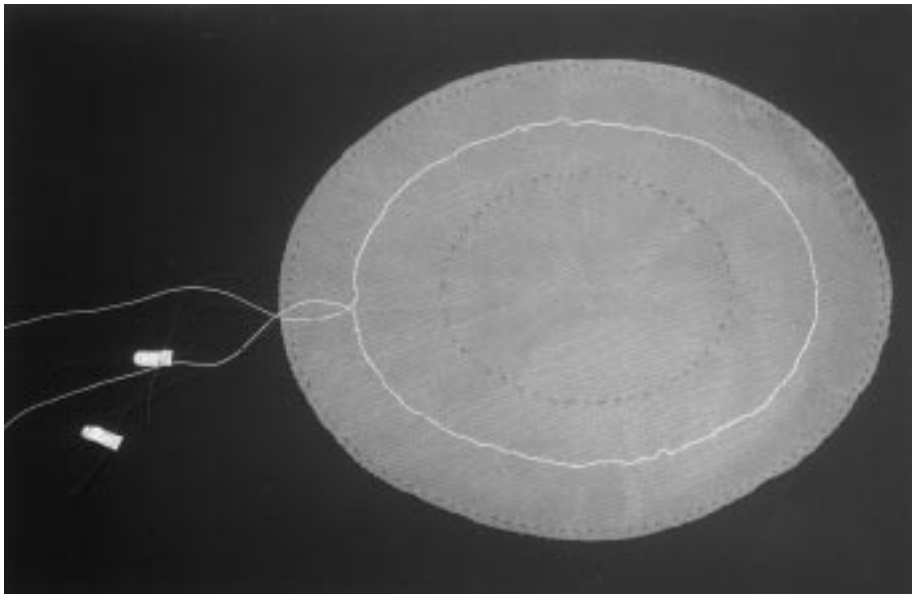

Fig 1 Resorbable polyactin net for the treatment of traumatic rupture of the spleen (30 cm diameter)

Results

Thirty of 31 patients showed no sign of bleeding after the procedure. One patient bled into the retroperitoneal space, and a splenectomy was performed as a safety precaution. One patient died as the result of serious brain injury. One patient underwent a re-laparotomy

because of a mechanical ileus caused by an adhesion after which a wound infection occurred. This same patient also had an incisional hernia, which was corrected 3 months later. During the procedure the spleen was inspected. The net was completely resorbed and the spleen, which had a somewhat thickened capsule, appeared intact. The haematological studies carried out indicated normal splenic function in all cases with the exception of one case of thrombocytosis. This patient received two nets because of extensive rupture of the spleen (grade IV-V), which resulted in a non-septic necrotic spleen. In the other patients, none of the postoperative liver and spleen scans showed any signs of infarction. Perisplenic effusion was found in 2 patients, which resorbed spontaneously. These patients had fever, in one case caused by pneumonia, and one case where no cause could be found. No infections occurred in the area of the spleens or laparotomy wound. The results are summarized in table II. Altogether there were only 2 serious spleen-related complications in 30 patients (6.7%).

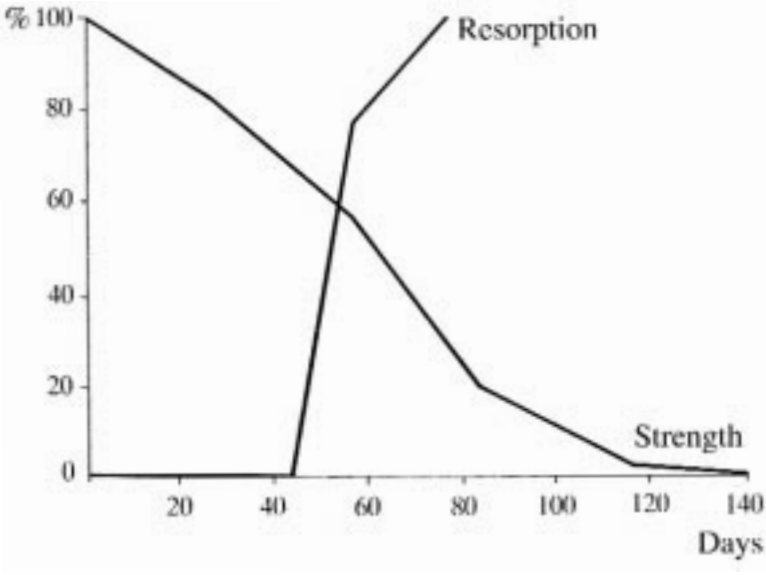

Fig 2 Histogram showing the tensile strength and the resorption time of the resorbable net.

Discussion

Kootstra extensively discussed the importance of salvaging the spleen as well as the available techniques[4]. We studied the clinical utility of one of these techniques, employing a resorbable net, which had previously been demonstrated to be effective in an animal model. If the resorbable net had not been available, these patients would have undergone

Table I Grading of splenic rupture in 31 patients according to Shackford[9], modified according to Kramer[10].

Grade I	Capsular tear, parenchyma intact	0
Grade II	Capsule tear into the parenchyma	6
Grade III	Parenchymal tear, not reaching hilus	11
Grade IV	Parenchymal tear reaching hilus	13
Grade V	Shattered spleen	1

splenectomy with autotransplantation of splenic tissue into the greater omentum[10]. By using this net we were able to save the spleens of 30 of our 31 patients in this study.

Lange et al. also reported good results with splenic wrapping in trauma patients A wrapping with an absorbable polyglycolic acid mesh was performed in 9 patients with grade II and III injuries of the spleen. There were no complications in these patients [11]. Subsequently, Rogers et al. reported good results without clinical problems using mesh splenorrhaphy in 14 patients[12]. Fingerhut et al. described their results of salvage by a splenic mesh in 17 patients in which 1 case required splenectomy afterwards and in which 2 patients developed non-infected perisplenic effusions[13]. In their opinion, the splenic mesh was safe and reliable. Two patients in our study also developed a perisplenic effusion that resolved spontaneously. This could have been related to the polylactine net, but resolved without further problems. In the case requiring 2 nets, it would probably have been better to perform a splenectomy immediately, as the initial damage was probably too extensive.

Søndenaa et al reported the results of wraps in 8 patients, but they had complications in 4 patients[14]. Two patients had pleural effusion, which resolved spontaneously, whereas rebleeding was present in 2 cases leading to splenectomy. In our opinion this rebleeding might have been avoided, as the net seemed not to have been applied in the proper way. The authors discussed that if treatment options should include more frequent use of the splenic mesh wrap, experience of the surgical team with treatment of splenic injury is necessary[14].

Wall, Hirshberg and Mattox wrote recently[15] that the most prevalent performance pitfall with splenic repair is failure to mobilise the injured spleen adequately. Hemisplenectomy, mesh wrapping, and other complex repairs are time consuming and should be viewed as special manoeuvres that require considerable experience.

Shackford and Molin stated in 1990 that, because of the importance of the spleen, it is appropriate that techniques for splenic preservation, either operative or nonoperative, should be understood and utilised, whenever appropriate, by all surgeons who manage splenic injury[16]. In each individual situation the surgeon must decide which technique is best for each patient.

Table II Results of the spleen-saving method for splenic rupture with an absorbable net.

Non-complicated procedures	27
Ileus	1
Non-septic necrosis	1
Death of brain injury	1
Precaution splenectomy (no splenic bleeding)	

Conclusion

On the basis of these results, we propose that a spleen-saving method should always be considered as a first option in the treatment of a traumatic rupture of the spleen before considering the option of a splenectomy, with or without an autotransplantation. The resorbable net has proven to be a major gain in this treatment.
The use of the absorbable net seems to be safe, but demanding operation technique, which requires experience in the treatment of splenic injury.

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CHAPTER 3

FC-RECEPTOR FUNCTION AFTER HUMAN SPLENIC AUTOTRANSPLANTATION

Leemans R*, Beekhuis H†, Timens W‡, Hauw The T§ and Klasen HJ*.
*Departments of *Surgery, †Nuclear Medicine, ‡Pathology and §Immunology, University Hospital Groningen, Groningen, The Netherlands*
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Summary

Mononuclear phagocytic function was studies using the Fc-receptor test in 24 patients who underwent splenectomy, ten of whom underwent splenic autotransplantation. All patients undergoing autotransplantation had mononuclear phagocyte system (MPS) activity at the transplantation sites. In eight of the 14 patients who did not undergo autotransplantation there was also scintigraphic MPS activity indicative of ectopic splenic tissue. Although the Fc-receptor test showed delayed and monoexponential blood clearance in all patients after splenectomy, there were no significant differences between the patient groups. Autotransplantation of small amounts of splenic tissue after splenectomy provides some MPS activity but is inadequate for blood clearance.

Introduction

Splenectomy results in a increased risk of sepsis, the so-called overwhelming post-splenectomy infection (OPSI) syndrome, which carries a high mortality rate, especially in children[1,2,3]. This syndrome is likely to be caused by a defect in the immune system after splenectomy[4,5,6].

The mononuclear phagocyte systems (MPSs) of the liver and spleen show clear differences in opsonization requirements. Whereas phagocytosis by the liver MPS requires adequate opsonization of antigen with immunoglobulin and/or complement, the splenic MPS is able to phagocytose poorly or non-opsonized antigens. This property is unique to the spleen[7], and is particularly important in the defence against thymus-independent type 2 antigens, such as those of Streptococcus pneumoniae, the main cause of OPSI[8]. Animal experiments have suggested that autotransplantation of splenic tissue (AST) after splenectomy restores this particular splenic MPS function[8,9,10]. The result of AST in humans has been evaluated using ^{99m}Tc colloid scintigraphy[11,12,13] and Cr-51 anti-rhesus factor antibody coated erythrocytes[14]. However, it remains unclear whether of not AST in humans restores MPS function after splenectomy.

A method of measuring MPS function has been developed which involves the clearance of radiolabeled rhesus-positive (intact) erythrocytes coated at low density with anti-rhesus antibody in patients with systemic lupus erythematosus[15] and Wegener's granulomatosis[16]. This Fc-receptor test is considered to provide a parameter of in vivo splenic MPS Fc-receptor function[17,18,19]. Low-level opsonization of the erythrocytes provides good uptake by the splenic Fc-receptors and reduced Fc-receptor uptake in the liver.

To evaluate whether autotransplanted splenic tissue may be capable of restoration of MPS function after splenectomy, this assay was employed in patients, with and without splenic autotransplantation after splenectomy, from whom the blood clearance and 'spleen' uptake curves were analysed.

Patients and methods

Patients

Splenectomy was performed either for trauma or as part of the surgical procedure during partial gastrectomy for peptic ulcer in 24 male patients, mean age 27.3 (range 15-55) years, none of whom had malignant or autoimmune disease. During the operation a thorough examination for ectopic splenic tissue was performed. When ectopic splenic tissue was found, the patients were excluded from the study.
In ten of these patients autotransplantation was performed after splenectomy: a 5x5x1-cm piece of splenic tissue (approximately 25 g) was cut into 2-3-mm cubes and implanted in the greater omentum, fixed with a purse-string suture and marked with a silver clip. In the remaining 14 patients autotransplantation was not performed.
The project was approved by the hospital medical ethics committee and informed consent was obtained from all patients.

Test procedures

To exclude any effects of trauma and/or operation the time interval between operation and the Fc-receptor test was at least 6 months (range 0.5-2.5 years).
The Fc-receptor test has been described previously15[15,16]. In brief, rhesus-positive red blood cells from a single donor were incubated with a 1:2 dilution of anti-rhesus (D) anti-serum. Small numbers of cells were stored in 10-ml aliquots at —80^{0}C. On the day of the clearance study the contents of one vial were labelled with ^{99m}Tc (efficiency rate greater than 90 per cent); the amount of radioactivity administered intravenously was 30 MBq. The upper abdominal region was scanned with a dual-headed gamma camera (Siemens ROTA, Des Plaines, Illinois, USA) and computer (DEC Gamma 11; Siemens) in 1-min frames over 1 h. Blood samples were taken after, 0, 1, 2, 3, 5, 8, 13, 18, 23 and 30 min and then every 10 min up to 2 h. The localisation of 'hot spots'on the scan was compared with abdominal radiographs, visualising the original localisation of the spleen transplants, marked with silver clips. The blood curve was examined using the compartment model16[16,20], the compartments studies being blood, spleen and liver. On entry into the spleen the ^{99m}Tc-labeled and immunoglobulin (Ig)G-coated erythrocytes are trapped either temporarily or permanently[14]. The assumption is made that reversibly trapped erythrocytes re-enter the blood by first-order kinetics. Because of the low level of opsonization the reversible pool is not situated in the liver but the spleen. This model would predict a biexponential clearance from the blood of the coated and labelled erythrocytes in case of a truly reversible compartment. Without any reversible compartment the clearance will be monoexponential[20].
In this study the shape of the whole blood curve over 2 h was investigated by the least squares fitting method with both a biexponential and a monoexponential function[16,20]. The sum of the squared deviations between the measured data points and the theoretical

functions in both fit procedures, the so-called χ^2 values, were used to describe the shape of the experimental curve. For a biexponential curve the χ^2 value for the fit is much smaller than for the monoexponential fit; the ratio of both χ^2 values, the so-called F value, is much less than unity. For a monoexponential curve the χ^2 values of both fits are equal, resulting in an F value near unity[16,20].

Statistics

For statistical analysis the non-parametrical Kruskal-Wallis test was used.

Results

Scintigraphy

In all patients undergoing splenic implantation (n=ten) scintigraphy showed a hot spot at the site where the splenic tissue had been reimplanted. The spot was small in all patients, corresponding with the small amount of implanted tissue.
In eight of the 14 patients who did not have an AST procedures a hot spot was found in the upper left abdomen; this spot was considered to represent ectopic splenic tissue. In the remaining six patients no such spot was found (no splenic tissue group). Scintigraphs of the patients are shown in figure 1.

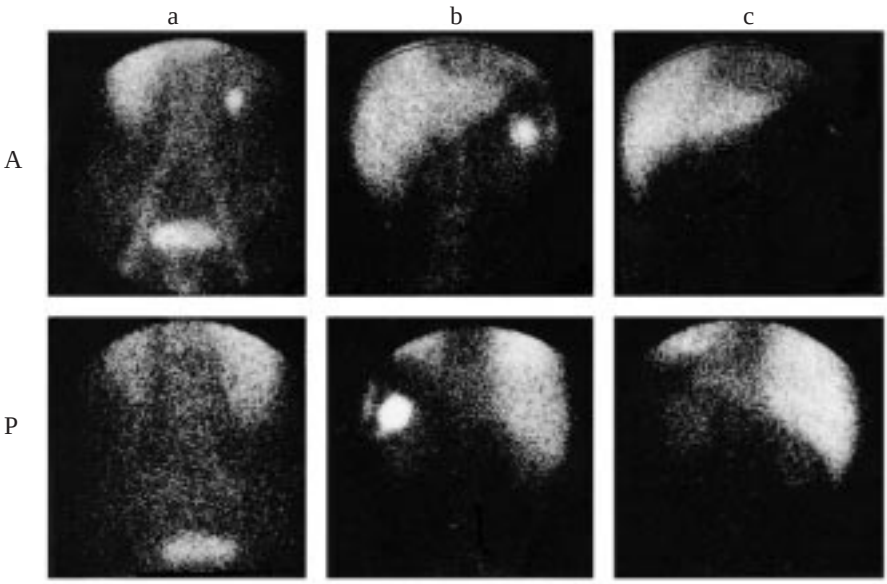

Fig. 1. Scintigrams of liver and spleen using 99mTc immunoglobulin (Ig)G coated erythrocytes; anterior (A) and posterior (P) view. a After splenectomy and autotransplantation of splenic tissue; b after splenectomy with ectopic splenic tissue; and c after splenectomy without ectopic splenic tissue

Blood clearance

The blood clearance curves of the coated and labelled.erythrocytes were abnormal compared with normal subjects ($P<0.05$). There were no significant differences between the two patient groups (*table I and fig. 2*). The curves did not show signs of a reversible compartment and were flat, with high χ^2 values for the monoexponential fit. The F values were high in all three groups (in biexponential curves they are low), and there were no statistically significant differences between the groups (*table II*).

Table I Percentage radioactivity of blood samples in the three patient groups

Time (min)	AST (%)	EST (%)	NST (%)
1	47(38)	53(35)	73(22)
2	87(18)	93(8)	97(4)
3	91(9)	95(6)	96(3)
5	94(9)	86(12)	90(9)
8	92(9)	86(10)	91(6)
13	90(10)	81(11)	88(6)
18	85(10)	78(12)	87(4)
23	86(10)	73(11)	87(7)
30	84(9)	73(11)	81(4)
40	80(9)	69(23)	78(7)
50	78(10)	67(10)	74(7)
60	77(9)	62(10)	70(8)
70	73(9)	62(13)	69(8)
80	69(7)	59(11)	70(7)
90	71(10)	59(14)	70(8)
100	67(9)	53(10)	67(8)
110	68(9)	53(13)	64(11)
120	68(12)	54(13)	62(9)

Values are mean(s.d.). AST, autotransplantation of splenic tissue; EST, ectopic splenic tissue; NST, no splenic tissue

Table II Results of Fc-receptor test in patients after splenectomy

Patient no. Half-life	(min)	Mono-exponential	Bi-exponential	F
AST				
1	152	1291	96	0.07
2	205	1043	720	0.69
3	370	1015	515	0.51
4	-	-	-	-
5	167	71	11	0.15
6	140	820	544	0.66
7	630	1584	-	1.0
8	136	428	341	0.80
9	186	462	99	0.21
10	263	2415	299	0.12
Mean (s.d.)	250 (152)			0,47 (0.32)
EST				
11	133	1354	739	0.59
12	374	986	772	0.78
13	111	942	364	0.39
14	178	555	169	0.64
15	187	1891	245	0.09
16	109	1137	83	0.22
17	107	1041	247	0.08
18	145	1861		0.13
Means (s.d.)	168 (83)			0.37 (0.26)
NST				
19	310	1388	1028	0.74
20	237	356	109	0.31
21	215	739	707	0.96
22	91	1365	793	0.58
23	197	1116	563	0.50
24	351	766	760	0.99
Means (s.d.)	234 (83)			0.68 (0.24)

AST, autotransplantation of splenic tissues; EST, ectopic splenic tissue;
NST, no splenic tissue, Reference values[16] are: half-life, 29 (range 11-56) min;
F=0-0.1; * χ^2 test

Discussion

The Fc-receptor test used in this study measures blood clearance and degree of reversible splenic pooling of low density IgG-coated red cells. A prolonged half-life, and splenic trapping which is either diminished or absent has been found in patients during active phases of systemic lupus erythematosus and Wegener's granulomatosis[15,16]. These results were interpreted as a saturation of the Fc-receptors in the spleen by circulating immune complexes, i.e. as decreased functional splenic MPS activity. Such a decrease can be interpreted as functional hyposplenism, but the effects of therapy, e.g. corticosteroids, on Fc-receptor function cannot be excluded. With this in mind, in the present study the Fc-receptor test was used in splenic tissue in order to investigate the MPS function of the reimplanted tissue.

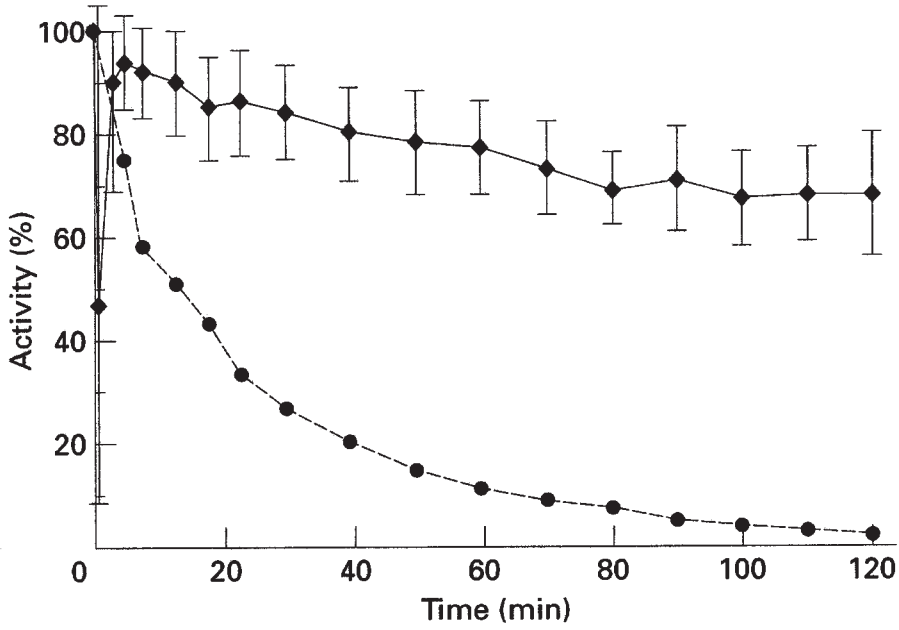

Fig. 2. Blood clearance curve (in percentage activity of 99mTc-labelled immunoglobulin (Ig)G-coated erythrocytes. Normal pattern with beixponential shape (---) and mean curve of the autotransplanted patients (——). Mean curves of the other patient groups were not significantly different

In the scintigraphy studies a hot spot was seen at the site of splenic autotransplantation suggesting the presence of functional MPS tissue. In eight patients without autotransplantation positive scintigraphy was also seen, showing either the presence of a residual accessory (or ectopic) spleen or possibly localised splenosis because all these patients had a disrupted spleen.

The blood clearance curves were not significantly different between splenectomy patients with and without autotransplantation, or with ectopic splenic tissue. The test measures reversible pooling of cells in the spleen. scintigraphy showed that 'phagocytic tissue' with functional 'splenic' Fc-receptors was present. In normal individuals the coated erythrocytes pool in the spleen resulting in a biexponential blood clearance (*fig. 2*), but in active phases of diseases such as systemic lupus erythematosus and Wegener's granulomatosis this pooling appeared to be absent resulting in a monoexponential blood clearance (*fig. 2*) with a prolonged half-life[15,16]. In a compartmental model with a reversible pool in the spleen this indicates strongly reduced splenic MPS function resulting in 'functional hyposplenism'. The results in this study indicate that, compared with patients with systemic lupus erythematosus and Wegener's granulomatosis, functional hyposplenism with respect to overall Fc-receptor function occurs after splenectomy. In autotransplanted patients the resulting 'spleen' was small (less than 10 per cent of the normal size) and the blood supply was much lower than that of the normal spleen.

The degree of antibody coating used in the present study was chosen because of the optimal splenic uptake; a higher degree of coating may result in a better liver uptake and shorter blood half-life[16], therefore this may bot be an adequate assay of splenic MPS function. Although restoration of general MPS activity is relevant, it should be appreciated that densely I-coated (well-opsonized) erythrocytes are not typical of the badly opsonized microbial antigens that are known to cause problems in OPSI.

In conclusion, scintigraphy with IgG-coated red cells showed a hot spot at the site of splenic implantation indicating the presence or Fc-receptor-bearing (phagocytic) cells, but the results of the analysis of the kinetics of these cells are not characteristic of adequate restoration of overall splenic Fc-receptor function. Based on the present study, autotransplantation of a small amount of splenic tissue after splenectomy cannot be considered sufficient to restore splenic MPS function, especially in relation to low-opsonized antigens. Therefore, at present medical prophylaxis is required to prevent OPSI in patients who have undergone splenectomy even when splenic autotransplantation has been performed. Further studies of MPS function after splenic autotransplantation may require procedures in which a considerable mass of well vascularised splenic tissue is trans planted in the hope that MPS function of the autotransplanted spleen will be preserved.

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Chapter 4

IMMUNE RESPONSE CAPACITY AFTER HUMAN SPLENIC AUTOTRANSPLANTATION: RESTORATION OF RESPONSE TO INDIVIDUAL PNEUMOCOCCAL VACCINE SUBTYPES

Leemans R*, Manson W‡,†, Snijder JAM‡, Smit JW#, Klasen HJ*, Hauw The T§, Timens W°.
*Departments of * Surgery, Medical Center Leeuwarden, Leeuwarden, and °Pathology,*
 # Haematology, § Clinical Immunology, and † Medical Microbiology, University of Groningen,
‡ Laboratory for Public Health, Groningen, The Netherlands,
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Summary

Objective: To evaluate features of general immune function, in particular the restoration of the humoral immune response to pneumococcal capsular polysaccharides, in humans undergoing a spleen autotransplantation after splenectomy because of trauma.
Background: After splenectomy, patients have an increased risk of overwhelming infection or sepsis involving encapsulated bacteria such as pneumococci. The value of human spleen autotransplantation after splenectomy because of trauma has long been questioned. Mononuclear phagocyte system function appeared to be similar to that in splenectomized persons. The presence of specific antipneumococcal antibodies would allow other parts of the mononuclear phagocyte system, such as those in the liver, to phagocytose opsonized bacteria.
Methods: Ten consecutive patients undergoing splenectomy followed by autotransplantation were compared with the next 14 consecutive patients undergoing splenectomy alone. After a minimum of 6 months, the patients were vaccinated with 23-valent pneumococcal vaccine. Blood samples were taken at the time of vaccination and after 3 and 6 weeks for anti-pneumococcal capsular polysaccharides IgM and IgG enzyme-linked immunosorbent assay against types 3, 4, 6, 9, 14, and 23. Splenic regrowth was evaluated by scintigraphy.
Results: Surprisingly, several of the nonautotransplanted patients showed scintigraphic activity, indicating the presence of either accessory spleens or traumatic seeding (splenosis). Significant antibody titer increases (more than twofold) were found for both IgM and IgG in the autotransplanted patients. Splenectomized-only patients showed no significant increase in Ig-levels in patients without splenic regrowth and partial improvement in patients with splenosis/accessory spleens.
Conclusions: Considering this significant anti-pneumococcal antibody rise, spleen autotransplants can be expected to permit an adequate humoral response to pneumococcal infections and presumably also to other TI-2 antigens, and to protect against overwhelming postsplenectomy infection or sepsis.

Introduction

The spleen has an important function in the host's response to infections by clearing polysaccharide encapsulated bacteria. This response involves the clearing from the bloodstream as well as the rapid production of specific antibodies against the polysaccharide antigens. After splenectomy, there is an increased risk of septic complications with a high mortality rate, especially in children. This is known as the "overwhelming post-splenectomy infection syndrome"[1,2].
The spleen is the major organ for the phagocytosis of low or nonopsonized particles; hence, after splenectomy there is a decrease in the capacity of the mononuclear phagocyte system. Another important function of the spleen is production of tuftsin, a stimulator of phagocytic activity. Therefore, we can expect that after splenectomy the phagocytic activity of the polymorphonuclear granulocytes is decreased[3,4]. Finally, the spleen provides a rapid primary immunologic reaction by the production of immunoglobulins

after initial contact with an antigen, leading to the elimination of the antigen. It has been proposed that the initiation of the primary immune response to polysaccharide antigens, including pneumococcal polysaccharides, is specifically related to the spleen, in particular to the marginal zone[5]. This implies that after splenectomy there will be an impaired antibody response in relation to pneumococcal vaccination[6].

Animal experiments have suggested that autotransplantation of splenic tissue (AST) after splenectomy might restore defects in the immune system[7,8,9]. Most human studies to confirm or disprove this have so far been confined to nonspecific splenic function tests[10,11]. In view of the highly specific functions of the spleen, this is unsatisfactory, and for further progress we must find new parameters testing real spleen-related immune functions[12]. An example of detailed, adequate testing of specific splenic functions was given in a case report describing a patient with extensive posttraumatic splenosis[13].

As an initial evaluation of immune capability after splenectomy with or without autotransplantation, we have determined the general humoral response capability. The function of the nonspecific immune system was evaluated using tests for phagocytosing and opsonization capacities[14].

The most important question to be answered in this study is whether the specific antibody response after pneumococcal vaccination in splenectomized patients will be better if AST is performed. We evaluated specific antibody responses after vaccination with polyvalent pneumococcal vaccine with respect to five pneumococcal subtypes in splenectomized patients with and without AST.

Materials and Methods

Patients

During a period of 4 years we studied 24 male patients with a mean age of 27.3 years (range 15 to 55 years) who had undergone splenectomy after traumatic rupture of the spleen or during partial gastrectomy for peptic ulceration. Splenectomy was carried out only when splenorrhaphy or other conservative therapy was not possible (University Hospital protocol). During the operation, a thorough examination for ectopic splenic tissue was performed. When ectopic tissue was found, the patient was excluded from the study.

To exclude the immediate effects of trauma or surgery, the period of time between the operation and the investigations related to the present study was 0.5 to 2.5 years. The subsequent clinical follow-up period varied from 5 to 8 years (mean 6.5). The study was organized prospectively, with the patients entered at the time of splenectomy. In the first 10 consecutive splenectomy patients, autotransplantation was performed; the next 14 consecutive patients, including some splenectomized patients from other hospitals, did not undergo the autotransplantation procedure. No selection was applied whatsoever. There were no differences in indications for splenectomy or otherwise between the groups in the different hospitals. The hospital's medical ethics committee approved this project and informed consent was obtained from all patients.

Spleen Autotransplantation

In 10 patients, autotransplantation was performed after splenectomy with 20 to 30 g splenic tissue. A slice measuring 5 x 5 x 1 cm, including part of the capsule, was taken from the spleen and divided with a scalpel into cubes of in general about 3 mm, with a maximum of 5 mm diameter. This autotransplant was then fixed in the greater omentum with a purse string suture, and the site was marked with a silver clip. In the remaining 14 patients, no autotransplantation was performed.
During their hospital stay, all patients received prophylactic antibiotics (cefuroxime).

Fc-Receptor Scintigraphy

In all patients, Fc-receptor scintigraphy with IgG-coated and 99mTc-labeled red cells was performed to check the mononuclear phagocyte system function and consequent presence of autotransplants. Because the red blood cells are coated with low-density immunoglobulin, this provides a low level of opsonization that causes a reversible pool of the labeled red cells in splenic tissue, when present, and not in the liver. In this way, a selective visualization of splenic tissue (red pulp mononuclear phagocyte system) is obtained. We described this method in more detail previously[15].
In short, the test procedure for the scintigram is as follows. Red cells from a single donor had previously been incubated with a suspension of 1:2 antirhesus (D) antiserum. Small numbers of these cells were stored in 10 ml ampules at -80°C.
On the day of the test, the contents of one ampule were labeled with ^{99m}Tc. The abdominal region was scanned with a dual-headed gamma camera and computer.

Phagocytosis and Opsonizing Capacity Test

Granulocytes were isolated, washed, and resuspended in Ca^{2+}-containing HEPES buffer at a final concentration of 15 x 10^6 cells/ml.
Staphylococcus aureus was cultured in BHI-broth and washed in phosphate-buffered saline. The bacteria were spectrophotometrically brought to a final concentration of 10^9 bacteria/ml in Ca^{2+}- and Mg^{2+}- containing HEPES buffer. A suspension was made of 1 ml HEPES with bacteria and 60 ml (3%) serum. Opsonization took place in the presence of Mg^{2+} and Ca^{2+} for proper complement activation both via the classical and alternative pathways. After the opsonization of the bacteria and after warming the granulocytes to 37°C, they were put together in a ratio of 2:1 to determine the phagocytosis in a spectrophotometer. Phagocytosis was measured during 30 minutes as an increase in light transmittance given as a percentage, compared with reference values.

Nitro Blue Tetrazolium Test

The nitroblue tetrazolium test is a standard test for evaluating the phagocytic function of

granulocytes[16,17]. A drop of blood is put on a slide prepared with endotoxin and incubated. After washing with saline, the granulocytes adhere to the glass. Subsequently, the slide is incubated with nitroblue tetrazolium and pooled serum of healthy donors. The active granulocytes reduce nitroblue tetrazolium to blue formazan after phagocytosis and can be counted. The result is given as the percentage of positive cells, counting 100 cells.

Phagocytosis Killing Test

Granulocytes were purified, washed in HEPES with saline, and put into a bacteriologic-grade 96-well incubation plate together with serum. Cultures of S. aureus were put in phosphate-buffered saline, and this suspension was added to the plate in a two-step dilution. After incubation at 37°C, 10 µl from each well was put into nutrient agar. After incubation, the well with >5 bacterial colony-forming units was considered the bactericidal titer and was used to measure the largest bacterial population that was eliminated by phagocytosis. The result is given as a percentage of the dilution, compared with granulocytes of healthy donors.

Helix pomatia Hemocyanin Test

This test is used to test general humoral immune capacity[19,20]. A vaccination of 1 mg Helix pomatia hemocyanin (HPH) is given subcutaneously and blood is taken before and 3 and 6 weeks after vaccination to determine specific antibodies IgG, IgA, and IgM. An indirect enzyme-linked immunosorbent assay (ELISA) technique is used. The results are given in arbitrary units. Patients with increased levels of specific anti-HPH levels on the day of immunization or abnormally high levels after 3 or 6 weeks were excluded from the test.

Immunoglobulin and Complement Levels

The levels of immunoglobulins and of C3, properdin factor B (PFB: C3 activator), and C4 were measured in an automatic nephelometer. The radial immunodiffusion technique was used for C1q, and C3d was measured by an ELISA with rabbit antihuman C1q- and C3d-antibodies. The reference values were obtained from healthy volunteers of the laboratory and checked with standard values. The immunoglobulin and complement levels were compared to the standards used by the laboratories in the University Hospital. All determinations of one test were performed at the same time.

Immunization

All patients were immunized after the splenectomy, with a lag time of >6 months to allow full regrowth of the transplanted splenic tissue.
Immunization was performed with 23-valent pneumococcal vaccine (Pneumovax®, Merck, West Point). Blood was taken on the day of immunization, and after 3 and 6 weeks

to measure of IgM and IgG against pneumococcal polysaccharide subtypes 1, 3, 4, 6, and 14 (Danish nomenclature) using an ELISA-technique, as previously described[21]. Purified pneumococcal polysaccharides of different subtypes were obtained from the American Type Culture Collection (Bethesda, MD).

ELISA-procedure

The ELISA was performed similar to previously described methods. 21 Wells of microtiter plates were coated with a 150-µl solution of each of the five pneumococcal polysaccharide types (1, 3, 4, 6, and 14) and in addition with Pneumovax; polysaccharides were used in a concentration of 5 µl/ml antigen dissolved in 0.1 M $NaHCO_3$ (pH 9.6) and incubated overnight at 4°C. After washing four times with 150 µl 0.9% NaCl containing 0.05 Tween 20 (Saline T), plates were freeze-dried and kept at 4°C until further use.
Measurements of IgG and IgM against the pneumococcal antigens were performed as follows. Patient and control sera (150 µl) were diluted 1:640 to 1:5120 for the IgG antibodies and 1:160 to 1:1280 for the IgM antibodies in 5 mM Tris-HCl buffer (pH 7.2) containing 0.05% Tween 20 (Tris T) and incubated for 90 minutes at 37°C. Subsequently the plates were washed five times with Saline T and incubated for 90 minutes with 150 µl peroxidase conjugated antihuman IgM (Nordic Immunology, Tilburg, The Netherlands). After incubation, the plates were washed five times with Saline T and were incubated for 45 minutes at 20°C with substrate (150 µl 0-phenylene diamine diluted in 0.025 phosphatase buffer [3 mg/ml] containing 0.02% H_2O_2, pH 6.3). The reaction was stopped by the addition of 50 µl 1N H_2SO_4. The absorbance was measured at 492 nm. The negative standard was a mixture of serum obtained from 3-month-old healthy babies, in whom pneumococcal infections were unlikely and residual maternal antibody was low.
The results are given in relative units, allowing determination of antibody increases after vaccination. The positive control was a mixture of sera of vaccinated volunteers.

Statistics

The incidence of patients with increases of antibody levels against pneumococcal polysaccharides of twofold or more were analyzed using Fisher's exact test. The levels of antibodies in the different groups were compared using the Mann-Whitney/Wilcoxon test.
The Kruskal-Wallis and Wilcoxon's nonparametrical tests were used for statistical analysis. $P<0.05$ was accepted as significant.

Results

Patients

No patient developed an overwhelming postsplenectomy infection syndrome during the period of investigation, nor was there any evidence of immune deprivation such as an

Table I. Results of Immunological Test Parameters in Patients after Splenectomy (Mean Values and One Standard Deviation)

Parameters	Reference	AST	EST	NST
POC-test (%)				
-opsonization	100±10	121±23	122±24	139±25
-phagocytosis	100±10	106±18	99±13	94±7
NBT-test (%)	>80	89.4±5.3	90.1±5.7	87.8±5.4
PK-test (%)	>96	96.3±4.0	97.7±3.9	99.0±0.4
HPH-test(units)				
IgG: 0 weeks	<1	0.5±1.2	1.0±1.0	0.2±0.4
3 weeks	>6	16±10	18±18	33±34
6 weeks	>6	21±20	15±16	22±21
IgA: 0 weeks	<1	0.2±0.4	0.3±0.7	0.4±0.5
3 weeks	>6	26±17	25±20	64±35
6 weeks	>6	25±28	14±11	39±30
IgM: 0 weeks	<1	4±3	3±2	4±6
3 weeks	>6	17±7	21±21	18±9
6 weeks	>6	16±8	18±14	18±1
Immunoglobulin (g/l)				
IgG	8.5-15	13.0±2.2	11.7±2.3	12.2±2.9
IgA	0.9-4.5	2.6±0.8	3.3±1.8	3.1±0.8
IgM	0.6-2.6	1.4±1.0	0.9±0.5	1.3±0.7
Complement				
C1q (mg/l)	100-250	103±4,6	100±3,9	101±5,3
C3 (g/l)	0.64-1.20	1.07±0.2	0.93±0.33	0.90±0.16
C3d (mg/l)	0.8-5.2	3.5±0.8	3.0±0.6	3.0±0.6
PFB (g/l)	0.19-0.40	0.32±0.05	0.30±0.03	0.35±0.03
C4 (g/l)	0.11-0.40	0.30±0.07	0.28±0.05	0.29±0.12

AST: patients with autotransplantation (n=10)
EST: ectopic splenic tissue (n=8)
NST: no splenic tissue (n=6)

increased general infection rate. There were no complications attributable to the presence of the autotransplants.
Based on the scintigraphic results, three groups of patients could be distinguished. The Fc-receptor scintigram showed a hot spot at the sites of autotransplantation grafts in the 10 patients (AST) in which this procedure had been performed. In 8 of the 14 patients who did not undergo AST, a hot spot on the scintigram suggested splenosis or ectopic splenic tissue (EST). These hot spots showed random localization, not similar to the autotransplanted tissue, at the site of the silver clip marking. Ectopic tissue present as accessory spleens was less likely because of the inspection during laparotomy, excluding patients in whom accessory splenic tissue could be identified. In six cases no activity was found on the abdominal scintigram except for the liver; this group was considered to have no splenic tissue (NST). The patients are comparable in terms of age, sex, and the time between splenectomy and immunization with the pneumococcal vaccine.

Immune Tests

All the phagocytosis activity tests showed normal results in relation to the reference values (see Table I page 75), and no significant differences were found between the patient groups.
Complement and immunoglobulins also showed normal values, without significant differences between the groups.

Vaccination

The results of the HPH-test showed values in the normal range for all three patient groups, without significant differences (see Table I page 75). One NST patient had a strong reaction on immunization; because the value (after 3 and 6 weeks, IgG 26 and 832, IgA 51 and 104, IgM 20 and 254 units) by far exceeded the mean plus two times the standard deviation, this was excluded from the mean values. Such incidental abnormal values have been reported previously[22].
The patients in the three groups showed no differences in the levels of specific IgG and IgM in the serum before vaccination for each of the tested serotypes. Six weeks after vaccination, most patients in the three subgroups showed a twofold increase of IgM antibodies against the whole vaccine (AST, 8/9; EST, 7/7; NST, 4/6).
The specific antibody responses against the different subtypes of Pneumovax® are listed in table II. There is a remarkable difference in the three groups with respect to the antibody reactions against the several serogroups. The AST patients showed a twofold rise of antibody titer against serogroups 1, 3, 4, and 14 for IgM antibodies, and 1 and 4 for IgG antibodies. The EST group showed a twofold increase in the IgM titer to types 3 and 4 and no rise in the IgG titers. The NST group showed neither an IgM nor an IgG rise to the tested antigens. Increase in antibody titers was the highest in the AST group, particularly with respect to IgM antibodies. After 3 weeks there was a significant increase in the IgM anti-

body response compared to the NST group to serotype 1, type 3, type 4, and to the total vaccine ($p<0.05$). Comparing the AST group with the EST group, there was a reduced response in the latter patients for type 4 ($p<0.02$). The EST group compared with the NST group showed an enhanced IgM response to type 3 ($p<0.05$).
Six weeks after vaccination, the differences in the IgM antibody response showed the same pattern. At this time there was a significant difference in the IgG antibody response for type 6 and type 14, AST versus NST; type 14, AST versus EST; and type 6, EST versus NST (all $p<0.05$).

Table II. Human Spleen Autotransplantation : Elisa Results of Specific Anti-pps Antibody after Pneumovax® 3 and 6 Weeks after Vaccination

	Subtype		**AST**			**EST**			**NST**	
	weeks	**0**	**3**	**6**	**0**	**3**	**6**	**0**	**3**	**6**
PPS1	IgG	39	65	130	54	58	56	45	52	57
	IgM	35	196	150	36	61	78	57	69	68
PPS3	IgG	16	16	16	25	18	18	15	15	15
	IgM	73	158	185	57	133	151	108	105	114
PPS4	IgG	72	250	300	81	139	111	84	112	119
	IgM	55	289	296	43	76	87	78	120	134
PPS6	IgG	97	97	121	109	127	116	108	116	150
	IgM	45	57	78	43	62	62	64	69	80
PPS14	IgG	223	343	351	181	230	280	196	226	276
	IgM	49	82	110	52	69	60	88	90	112

AST: patients with autotransplantation (n=9)
EST: ectopic splenic tissue (n=7)
NST: no splenic tissue (n=6)

Twofold or more rises are underlined.

Discussion

In a nontrauma situation, it is clear that pneumococcal vaccination should be performed before splenectomy. The usefulness of vaccination after splenectomy in case of trauma is often disputed. Previous animal studies reported that splenectomized subjects had a better antipneumococcal capsular polysaccharide antibody response to pneumococcal vaccination after spleen autotransplantation[23]. Another animal study demonstrated that autotransplantation improved the immunoglobulin levels after reimmunization[24]. Although the positive effect of spleen autotransplantation on restoration of immune functions in humans has been subject to doubt, the present study demonstrated that a better antibody response to pneumococcal vaccine was found in patients after splenectomy if an autotransplantation was performed, in agreement with an earlier case report on a patient with extensive splenosis[13].

In humans, deliberate spleen autotransplantation is performed in the highly vascularized greater omentum. Some reviews[9,11,25] have found that in humans the total volume of the autotransplanted spleen, not the individual fragment size, is critical in relation to the extent of necrosis, which is known to occur in the initial phase after autotransplantation. In fact, the reticular structure remains intact in a rim inside the preserved outer area of the autotransplant. We believe this reticular structure is important in the specific anatomy of the spleen, especially with respect to the sinusoidal structure of the marginal zone; therefore, we maintain this structure by using very small fragments or even putting the tissue through a mesh. Because we tend to agree that limiting the total volume is critical, using fragments of sufficient size, again theoretically, could serve as a "homing" structure for cellular outgrowth/repopulation of the spleen fragments.

In our study, we did not find a decrease in polymorphonuclear granulocyte function after splenectomy because of the lack of the normally present stimulating effect of tuftsin[3,4]: we found no difference in polymorphonuclear granulocyte function after splenectomy with or without AST or EST. This situation has also been described by others[26,27]. This difference may be related to the difference in time intervals between splenectomy and the functional studies, which in most reports were quite short. In our study this lag time was deliberately increased to avoid the immediate effects of trauma and surgery and to allow any compensatory mechanisms to develop.

Testing the primary immunologic response with HPH did not show any differences between the patient groups: all results were within the normal range. However, this test is not specifically spleen-dependent, and it seems very likely that the extrasplenic B-cell compartments compensate for the loss of the splenic B-cells.

In 8 of the 14 patients who did not have autotransplants, there was activity on the scintigram suggesting the presence of EST. This activity, observed at different sites in the abdomen, may result from the presence of accessory spleens or may be more likely due to splenosis from seeded (autotransplanted) splenic cells after splenic rupture[13,28,29,30]. There is a strong possibility that such "born-again" spleens will provide immune protection after splenectomy; this was extensively discussed by Hathaway et al.[13]. However, failure of

EST to prevent fatal pneumococcal septicemia after splenectomy has been described[31]. None of the patients in this limited survey showed any clinical evidence of immune deficiency in the follow-up period. The importance of the presence of EST is not yet clear and deserves more study.

It is likely that pneumococcal infections occur only in persons who lack antibodies to the capsular polysaccharide of the colonizing Streptococcus pneumoniae. In fact, studies conducted with cell wall polysaccharide antigens to measure an antibody response do not distinguish between antibodies directed to capsular polysaccharides and to cell wall polysaccharide antigens. Musher et al132 suggested using a specific ELISA with an absorption step to remove antibodies to cell wall polysaccharide antigens to specify the antibody response to the specific polysaccharides[32]. Nevertheless, in our ELISA, we used capsular polysaccharide antigens and were able to demonstrate an antibody response to the polysaccharides in healthy vaccinated individuals[21], and in some patients (AST group to subtype 1, 3, 4, and 14).

From our results, it appears that AST patients had a significantly better IgM response to serotypes 1 and 14 in comparison with EST patients, whereas for serotype 3 and 4 there was no substantial difference in the IgM response. It has been proposed previously that EST in humans does not normalize the altered antibody responses after splenectomy[33]; the limiting factor in these cases is not yet clear. One possibility is that either the reticular structure present in these accidentally implanted fragments or the blood supply is insufficient to allow restoration of adequate lymphoid tissue[34]. In the NST-group there was no significantly improved reaction at all.

The fact that the most important increase in immunoglobulin was of the IgM-class indicates that the respective pneumococcal capsular polysaccharide subtypes were encountered for the first time and that the spleen probably is essential in eliciting an adequate primary humoral response.

Because with splenectomy the restoration of clearance by autotransplantation is insufficient[15], restoration of the humoral response becomes even more important, allowing good opsonization of pneumococcal capsular polysaccharides, which enables clearance by the liver's mononuclear phagocyte system. When small amounts of regenerated autotransplanted spleen are present, with adequate response capacities to TI-2 antigen, specific antibodies will be formed. These antibodies will cause opsonization of the antigen (encapsulated bacteria) and, although the phagocytosing capacity of the splenic fragments will not play an important role, the cells of the mononuclear phagocyte system in the liver are then perfectly capable of removing these well- opsonized particles.

In conclusion, this study shows a surprising effect of AST after splenectomy on specific antibody responses after pneumococcal vaccination. Although it is not yet clear whether complete protection against all pneumococcal subtypes can be obtained, spleen autotransplantation may be expected to help limit the risk of overwhelming postsplenectomy infection syndrome. This study demonstrates that spleen autotransplantation can play a role in the management of severe splenic injury in which splenectomy is inevitable, particularly when followed by vaccination with a polyvalent pneumococcal vaccine. Our

results may provide a foundation for more extensive clinical studies with longer-term follow-up to put our findings in a useful clinical context. Further, the use of a panel of specific spleen-dependent antigens, such as different pneumococcal polysaccharide subtypes, can provide a procedure for testing functional splenic immune response capacity (e.g. to test the success of an autotransplantation procedure).

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Chapter 5

SPLEEN AUTOTRANSPLANTATION PROVIDES RESTORATION OF FUNCTIONAL SPLENIC LYMPHOID COMPARTMENTS AND IMPROVES THE HUMORAL IMMUNE RESPONSE TO PNEUMOCOCCAL POLYSACCHARIDE VACCINE

Leemans R*, Harms G†, Rijkers GT‡, Timens W.†

**Department of Surgery, Medical Centre, Leeuwarden, The Netherlands; † Department of Pathology, University Hospital, University of Groningen, NL-9700 RB Groningen, The Netherlands; ‡ Department of Immunology, University Hospital for Children and Youth "Het Wilhelmina Kinderziekenhuis", Utrecht, The Netherlands.*

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Summary

After splenectomy, patients have an increased risk of overwhelming post-splenectomy infection or sepsis involving encapsulated bacteria such as pneumococs. The value of spleen autotransplantation after splenectomy because of trauma has long been questioned. Much attention has been given to the restoration of mononuclear phagocyte system (MPS) function, which appeared to be similar to that of splenectomized individuals. The presence of specific anti-pneumococcal antibodies may enhance phagocytosis of opsonized bacteria by other parts of the MPS, as present in the liver. Therefore, in the present study we have evaluated the restoration of the humoral immune response after spleen autotransplantation, especially to pneumococcal capsular polysaccharides (PPS).
Wistar rats were divided into three groups that were operated as follows: splenectomy, splenectomy followed by autotransplantation, and sham operation. After 12 weeks the rats were vaccinated with 23-valent pneumococcal vaccine. Blood samples were taken after 3 days, 3 and 6 weeks for anti-PPS IgM and IgG ELISA against types 3, 4, 6, 9, 14 and 23. In addition, immunohistological studies were performed on the autotransplants.
Significant antibody titre rises were found in a main proportion of the autotransplanted rats, comparable with sham-operated rats. Splenectomized rats showed as well significantly lower increase in Ig-levels, as significant differences in proportion of rats showing a minimum 2-fold increase of antibody level, considered to represent an adequate response. The titres were highest 3 days after vaccination. Immunohistochemical studies demonstrated structurally functional autotransplants including an intact marginal zone.
Considering this significant anti-pneumococcal antibody response, spleen autotransplants can be expected to enable an improved humoral response to PPS, and to contribute to protection against OPSI after splenectomy.

Introduction

After splenectomy, patients have an increased risk of overwhelming post-splenectomy infection (OPSI) or sepsis (OPSS). Such an infection has a high risk of mortality, especially in children[1,2]. Fatal OPSI is in most cases caused by pneumococci, the capsular polysaccharides of which are considered to be T-cell-independent type 2 (TI-2) antigens. It is known that the initiation of the antibody response to such antigens depends on the presence of splenic tissue[3], in particular a functional marginal zone B-cell compartment[4,5]. Although spleen autotransplantation has been performed in an attempt to restore normal spleen immune function, until now it has not been known whether autotransplants of splenic tissue are capable of initiating a primary humoral reaction to TI-2 antigens.
In animal experiments, focusing on the influence of the marginal zone of splenic transplants on the antibody response to TI-2 antigens, the restoration of the antibody titres correlates with the return of B-cells, finally reaching titres indistinguishable from those of normal mice[6,7]. In a recent study, it was shown that autotransplanted tissue in newborn rats leads to a well-developed marginal zone and that this regenerated tissue provides

significant protection against pneumococcal infections introduced via the respiratory tract[8]. In most human studies the function of autotransplants was only tested by functional evaluation of the mononuclear phagocyte system (MPS)[9,10,11]. An example of detailed, adequate testing of specific splenic functions was given in a case report describing a patient with extensive post-traumatic splenosis[12]. In most animal experiments using pneumococci or Pneumovax® only general antibody titres were evaluated, and not subtype specific antibody titres[9,13]. The drawback of such an approach is that highly immunogenic PPS-types may elicit a considerable response, thereby masking potential non-responsiveness to other, less immunogenic types. Subsequently, the risk of OPSI in this situation may still be present for the latter PPS-types.

In the present study we investigated the effect of splenic autotransplants on the antibody responses to individual pneumococcal capsular polysaccharides of types 3, 4, 6, 9, 14 and 23. Antibody responses after vaccination with a pneumococcal capsular polysaccharide vaccine were compared in sham-operated, splenectomized and spleen-autotransplanted animals, respectively. To ensure full regeneration of white pulp in the splenic transplants, vaccination was performed 12 weeks after operation.

Materials and methods

Animals and operation

Male Wistar rats were housed under standard laboratory conditions on a 12-h light and dark cycle. They were fed with standard laboratory rat food (Hope Farms, Inc, Woerden, The Netherlands) and tap water ad libitum. Rats with a body weight of approximately 200 gr. of about three months of age were used for all experiments.
Sham operation (n = 10), splenectomy (n = 10) and splenic autotransplantation (n = 10) were performed as described by Pabst et al.[14]. In short, all operations were performed via an upper midline incision, under ether anaesthesia and clean but not sterile conditions. In splenic autotransplantation the spleen was removed and half of the spleen tissue was cut into pieces of approximately 1 mm^3, which were sutured into an omental pouch. The abdomen was closed in two layers.

Vaccination

For vaccination we used a 23-valent pneumococcal vaccine (Pneumovax®, Merck, Sharp, and Dohme, West Point, PA, USA) which contains the PPS types 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F. The sham operated, splenectomized, and spleen-autotransplanted rats, (for all n=10) were vaccinated 12 weeks after operation. Vaccination was performed by intramuscular injection in the left hind leg with one dose (0.5 Fl) of Pneumovax®.

Before vaccination (day 0) and 3 days, 3 weeks and 6 weeks after vaccination 500 µl blood was taken from the retro-orbital plexus under mild anaesthesia.
Six weeks after vaccination the animals were sacrificed and spleen or spleen autotransplants, were obtained at autopsy. The spleens and the autotransplants were weighed and tissue blocks were immediately frozen by immersion in liquid isopentane (cooled in a freezer to -80°C) and stored in a freezer at -80°C until sectioning.

Anti-PPS ELISA

To detect the anti-PPS 3, 4, 6, 9, 14 and 23 IgM and IgG antibody titres in serum, a sandwich enzyme-linked immunosorbent assay was used as described previously[15]. In short, ELISA plates were coated overnight at 37°C with 10 µg of PPS subtypes per ml in 0.9% NaCl. Pooled serum from all sham-operated animals (n=10), immunised with Pneumovax® (day 21) was used as an internal reference and assigned 100 U/ml antibody for all serotypes. To determine the anti-PPS concentrations in a given sample, serial dilutions were titrated into the plate. Adsorption with pneumococcal cell wall polysaccharide (CPS) was carried out by incubating serum samples overnight at 4°C with 50 µg of CPS (State Seruminstitute, Copenhagen, Denmark) per ml. The ELISA was processed by adding peroxidase-conjugated goat anti-rat IgM or IgG (Tago, Burlingame, Calif.) and incubated for 3 h at 37°C. Subsequently, the wells were allowed to react with 1.0 ml of 5.5 mM O-phenylenediamine-0.015% H_2O_2 in citrate phosphate buffer (pH 5). The yellow-brown reaction product was measured after 10 to 15 min at 450 nm with an ELISA reader.

Immunohistochemistry

To detect the different injected PPS-types[16], we used group or type specific rabbit anti-pneumococcal polysaccharide (anti-PPS) antibodies (State Seruminstitute, Copenhagen, Denmark). As second step antibody we used a peroxidase conjugated Swine anti-rabbit immunoglobulin (SAR^{per}; Dakopatts (Glostrup, Denmark). Kupffer cells in the liver and red pulp macrophages in the spleen were stained using the mAb ED2, marginal zone macrophages and metallophils in the spleen using mAb ED3[17] and as a B-lymphocyte marker the anti-IgM mAb His40[18].
Cryostat sections (4 µm) were air dried for 20 min, fixed for 10 min in acetone, air dried and washed in phosphate buffered saline (PBS) pH 7.4, for 5 min. Next, sections were incubated for 30 min with an appropriately diluted type or group specific anti-PPS antibody directed against PPS-type 3 ,4 ,6 ,9 or 14 with 5% normal rat serum (NRS). Subsequently, sections were incubated with SAR^{per} diluted 1:20 in PBS with 5% NRS for 15 min. The peroxidase activity was visualised using 3-amino-9-ethylcarbazole (AEC) and H_2O_2 as a reagent. Finally, sections were counterstained in Mayer's hematoxylin and embedded in Kaiser's glycerin-gelatin.

Statistical analysis

The incidence of rats with ≥ twofold rises of the antibody levels against pneumococcal polysaccharides were analysed with the Fisher exact test. The increase of levels of the antibodies in the different groups was compared using the Kruskal-Wallis and Mann-Whitney test. $P < 0.05$ was taken as significant.

Results

Immunohistology of autotransplanted splenic tissue

Eighteen weeks after autotransplantation the splenic transplants showed a reduced weight. The weight of the transplants was about 18% of the spleen weight in sham operated age matched rats. As about half of the spleen was transplanted, this equals approximately 36%

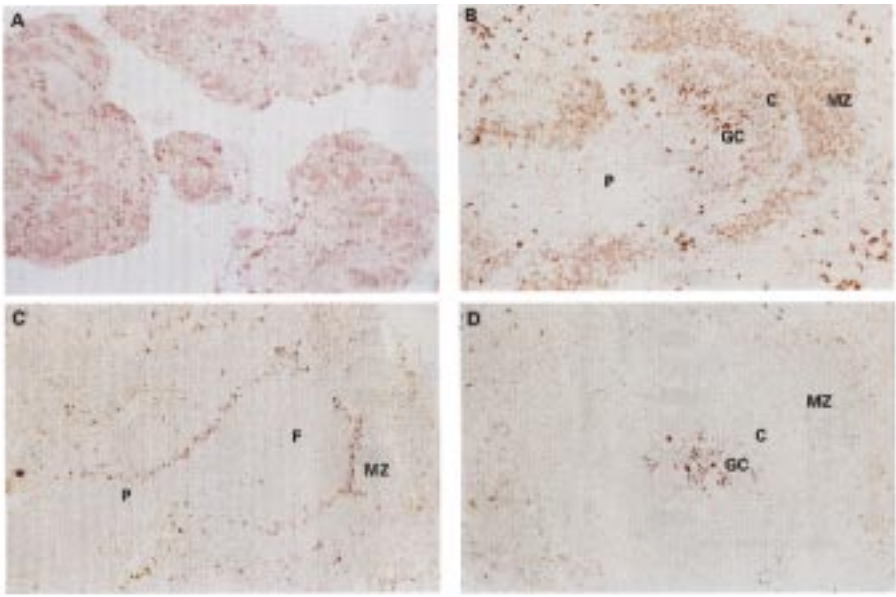


Fig 1. Microphotographs (all immunoperoxidase staining) demonstrating restoration of spleen lymphoid compartments including the functionally most relevant area, the marginal zone (12 weeks after autotransplantation procedure). A. all B-cell areas stained with IgM antibody His40: overview (x40) and B. magnification (x 100); C. marginal zone macrophages stained with ED3 (x 100). D. Staining for PPS1: localisation in a dendritic pattern in the germinal centre; presumably on follicular dendritic cells. (X 100).
C = lymphocyte corona; GC = germinal centre; F = follicle; MZ = marginal zone; P = periarteriolar lymphocyte sheath

of the originally implanted tissue weight. A clear demarcation of red and white pulp was seen and the latter was positioned directly beneath the capsule. Central parts of the transplants still contained fibrotic tissue. A clear compartmentalisation in marginal zone, mantle zone and germinal centre was detected with the anti-IgM mAb staining all B lymphocytes, and ED3 positive macrophages were present in the marginal zone. PPS-type 3, 4 and 9 but not PPS-type 6 and 14 were detected in the germinal centres in most of the splenic transplants. The PPS were localised in a pattern consistent with localisation on follicular dendritic cells (FDC). None of the PPS types localised in the marginal zone of the transplants, consistent with earlier kinetic studies, demonstrating that PPS localise in vivo in the marginal zone at an early time point[16], with a subsequent shift in localisation to the follicle/germinal centre showing (from 3 days after immunisation) a dendritic pattern. The above findings are illustrated in figure 1 (*page 89*).

Antibody responses

The IgM and IgG anti-PPS antibody response was studied in groups of animals that were sham operated, splenectomized, or splenectomized and autotransplanted. IgM and IgG anti-PPS antibody titres of the experimental groups in serum withdrawn before immunisation did not differ significantly from antibody titres in naive, non-immunised rats. Day 0 was considered baseline for each group, and for the other time points (3, 21, and 42 days after vaccination) the increase of PPS-type specific Ig level was determined in comparison to this baseline level.
In figure 2 the mean values for both IgM and IgG are given for each time point relative to baseline levels including standard deviations.
In sham-operated rats, immunisation with Pneumovax® induced a more than two-fold increase in both IgM and IgG antibody titres against all pneumococcal serotypes tested. The highest antibody titres were observed at day 3 after immunisation. At day 21, IgM antibody titres against most serotypes had dropped to almost pre-immunisation levels while IgG titres against serotypes 3, 4, and 9 still were higher than before immunisation (compared with baseline: 2.1-, 3.3- and 3.7-fold increase, respectively).

Fig 2. (page 91-92-93)

Graphical representation of increase in PPS type-specific IgM and IgG between day 0 (day of vaccination) and A. day 3, B. day 21 and C. day 42, respectively. A. * increase in immunoglobulin level significantly lower than sham-operated rats (* $p<0.05$; ** $p<0.01$). In B. and C. IgM levels are all below a mean of 2-fold increase (minimum of 2-fold increase is considered clinically relevant). For IgG at 21 days only for PPS9 is a relevant mean increase observed for the autotransplant group, not different from the sham operated group, whereas the splenectomy group has significant lower increase ($p<0.05$). For the other PPS no relevant increase was observed for either the splenectomy or the autotransplant group (the seemingly high increase for PPS23 was due to very low zero values for this PPS).

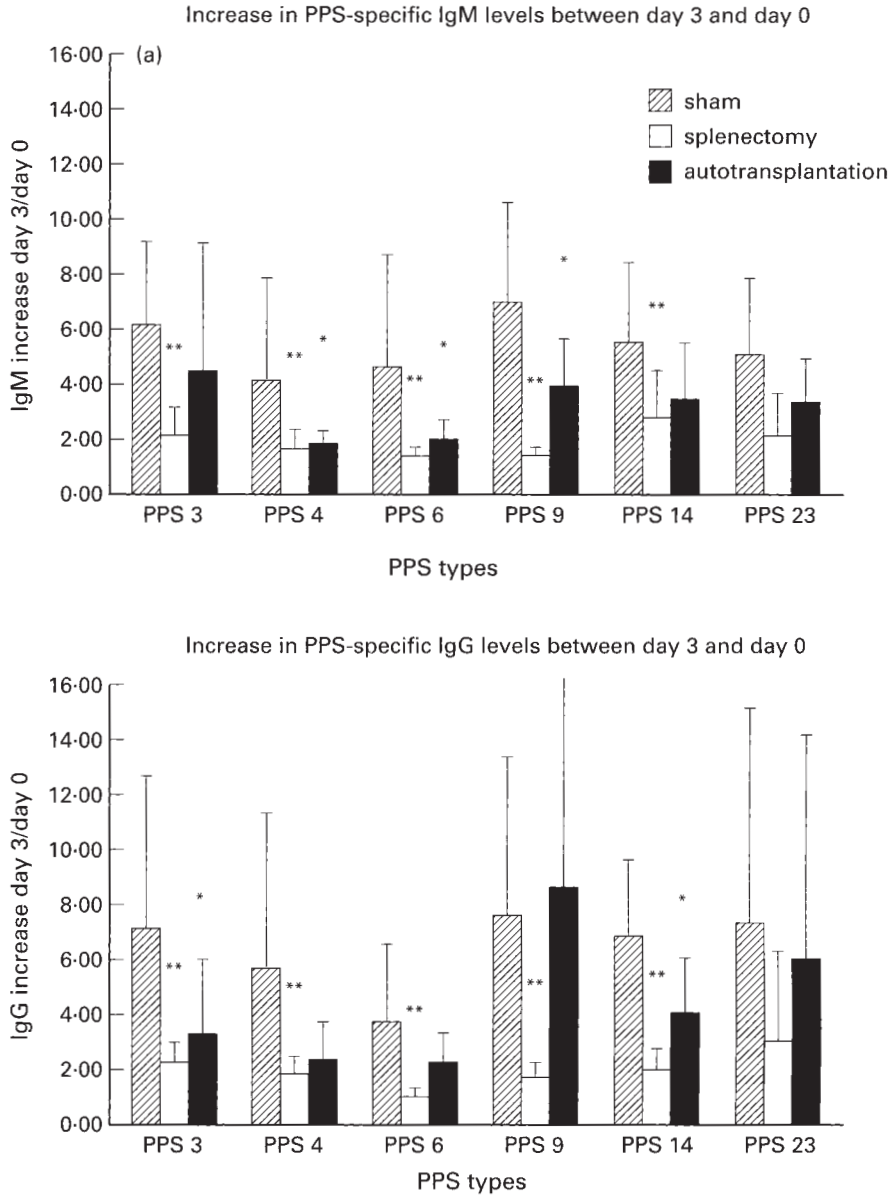
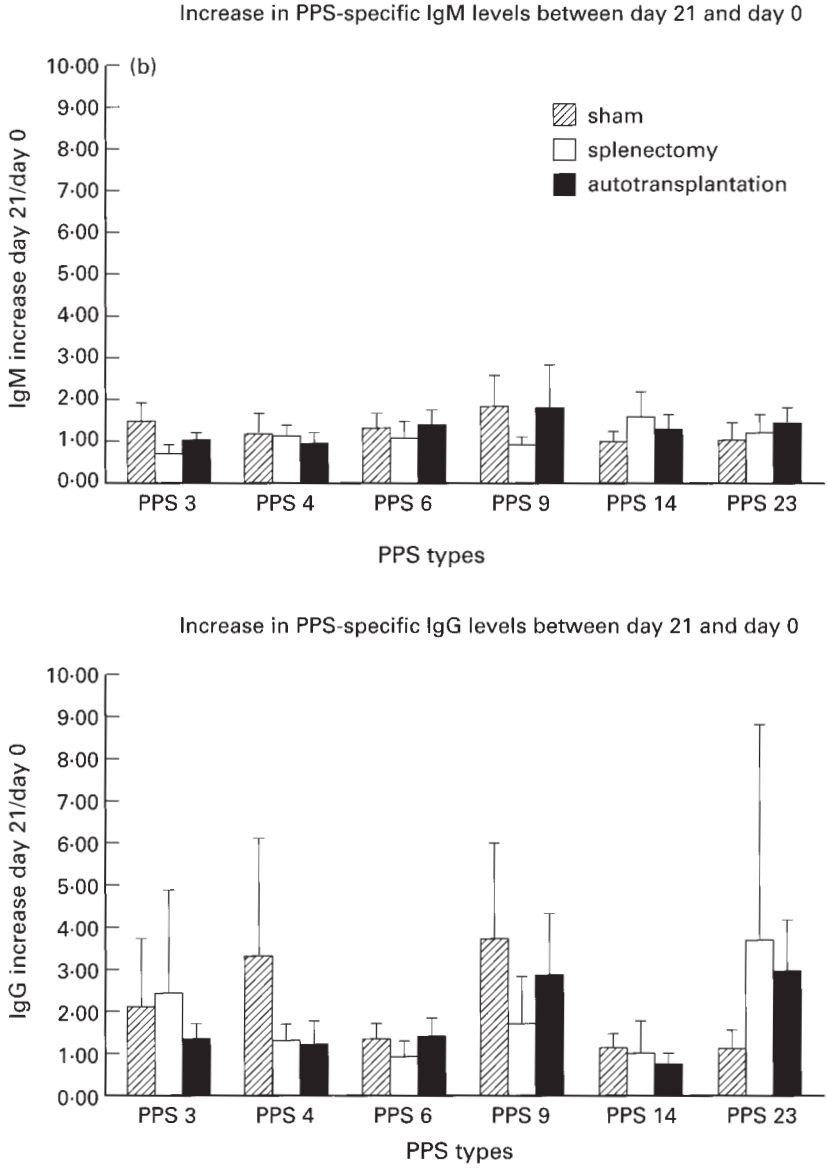

Increase in PPS-specific IgM levels between day 3 and day 0
(a)
sham
splenectomy
autotransplantation
IgM increase day 3/day 0
16·00
14·00
12·00
10·00
8·00
6·00
4·00
2·00
0·00
PPS 3
PPS 4
PPS 6
PPS 9
PPS 14
PPS 23
PPS types
Increase in PPS-specific IgG levels between day 3 and day 0
IgG increase day 3/day 0
16·00
14·00
12·00
10·00
8·00
6·00
4·00
2·00
0·00
PPS 3
PPS 4
PPS 6
PPS 9
PPS 14
PPS 23
PPS types

Fig .2A


Increase in PPS-specific IgM levels between day 21 and day 0
(b)
sham
splenectomy
autotransplantation
IgM increase day 21/day 0
10·00
9·00
8·00
7·00
6·00
5·00
4·00
3·00
2·00
1·00
0·00
PPS 3
PPS 4
PPS 6
PPS 9
PPS 14
PPS 23
PPS types
Increase in PPS-specific IgG levels between day 21 and day 0
IgG increase day 21/day 0
10·00
9·00
8·00
7·00
6·00
5·00
4·00
3·00
2·00
1·00
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PPS 3
PPS 4
PPS 6
PPS 9
PPS 14
PPS 23
PPS types

Fig. 2B

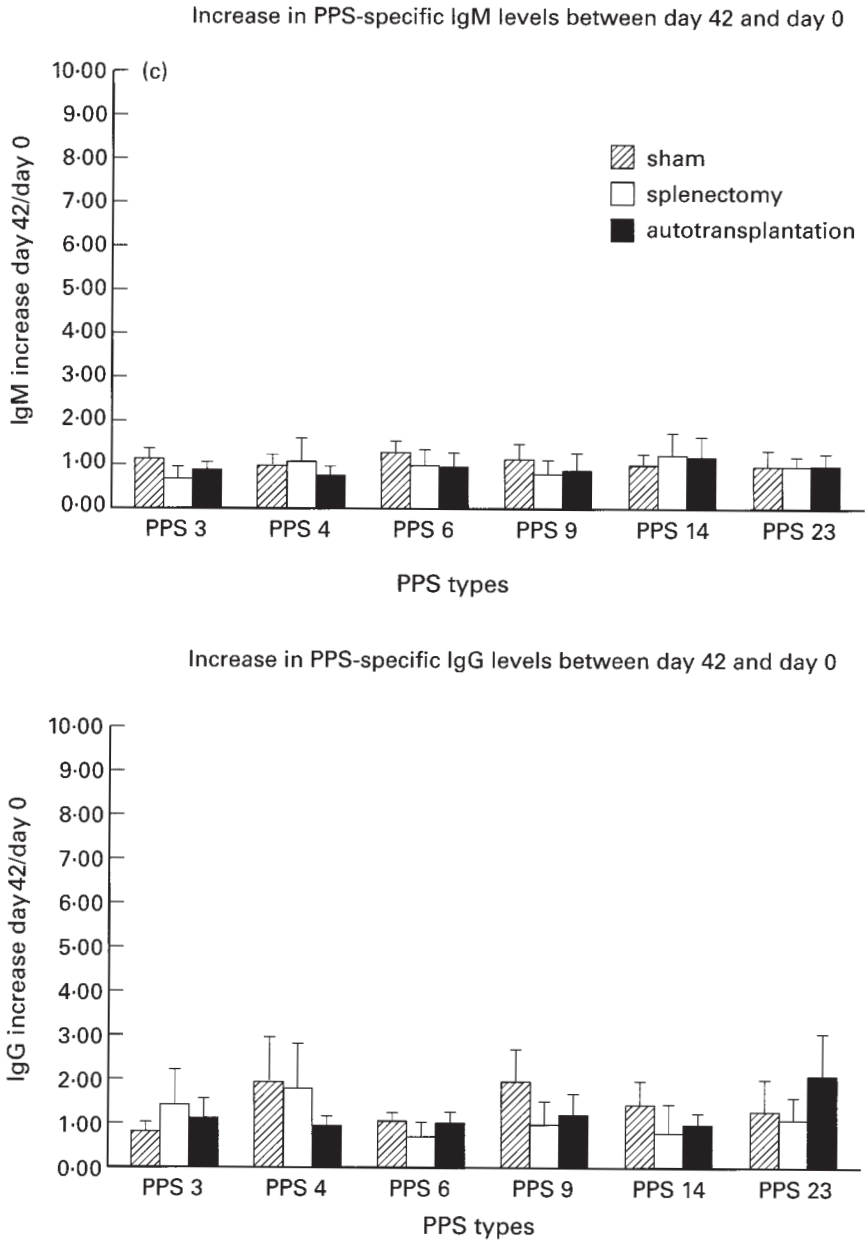
Increase in PPS-specific IgM levels between day 42 and day 0
(c)
IgM increase day 42/day 0
10·00
9·00
8·00
7·00
6·00
5·00
4·00
3·00
2·00
1·00
0·00
sham
splenectomy
autotransplantation
PPS 3
PPS 4
PPS 6
PPS 9
PPS 14
PPS 23
PPS types
Increase in PPS-specific IgG levels between day 42 and day 0
IgG increase day 42/day 0
10·00
9·00
8·00
7·00
6·00
5·00
4·00
3·00
2·00
1·00
0·00
PPS 3
PPS 4
PPS 6
PPS 9
PPS 14
PPS 23
PPS types

Fig .2C

In accordance with previous publications we found that splenectomy had a clearly negative effect on the anti-polysaccharide antibody response. At day 3 after immunisation in splenectomized rats compared with sham operated rats, a significant lower antibody titre was observed for all PPS types except PPS type 23 for both IgM and IgG (*fig. 2*). Splenectomy, followed by autotransplantation can partly rescue the antibody response to Pneumovax® vaccination: at day 3 IgM response to PPS 3, 14 and 23, and IgG antibody responses to pneumococcal serotypes 4, 6, 9, and 23 were similar in the autotransplantation group as compared with the sham-operated group.

When evaluating the number of rats in each group reaching ≥ two-fold increase in immunoglobulin titres, compared with the sham-operated group (*fig. 3*), both at day 3 and day 21, the splenectomy group showed a significant lower relative number of adequate responders for all PPS types except PPS14 for IgM and for all but PPS3 for IgG ($p < 0.05$).

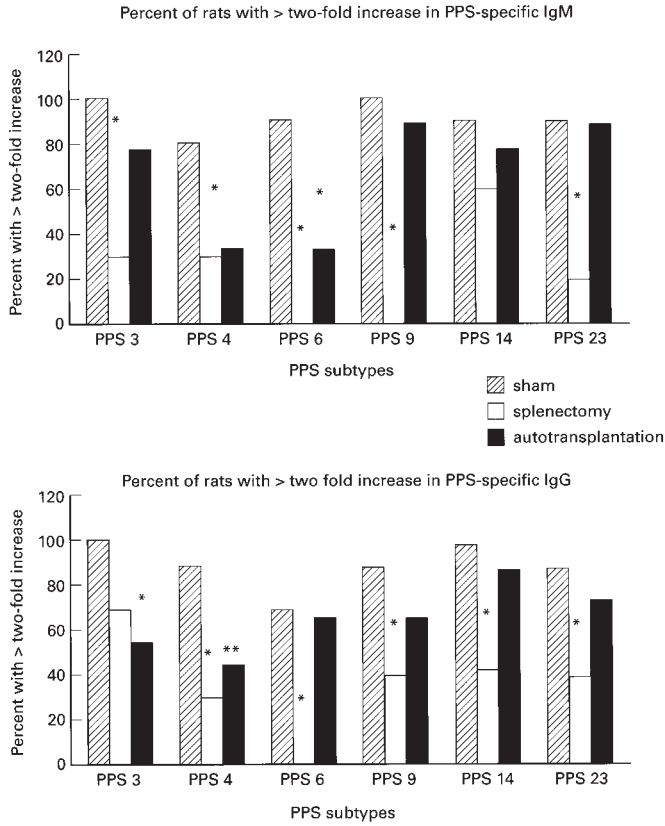

Fig 3. Graphical representation of number of rats per group with minimum of 2-fold increase in PPS type-specific IgM and IgG between day 0 (day of vaccination) and day 3. (Significant lower numbers compared to sham-operated rats: * $p<0.05$; # $p= 0.05$)

In contrast, for the autotransplanted group for IgM only for PPS4 and 6 and for IgG only for PPS3 and 4 was a lower number of responders found, both at day 3 and day 21. No other immunoglobulin titres were significantly different from the sham operated group. At day 42 only individual rats in the splenectomy and autotransplantation group showed more than two-fold increase in immunoglobulin titre, whereas the sham operated rats showed >2-fold responses for IgG levels for PPS4 (three rats), PPS9 (five rats) and PPS14 (two rats).

Discussion

Reimplantation of splenic tissue has been postulated to account for a lower incidence of OPSI among patients splenectomized for trauma[8,19]. However, the efficacy of splenic autotransplantation in restoring the immune response after splenectomy has not yet been established firmly. Some studies support the notion that immunisation combined with autotransplantation leads to higher antibody levels and may therefore offer a survival benefit[20]. In this study it is demonstrated that autotransplantation followed by vaccination confers the ability to mount an improved IgM and IgG antibody response to pneumococcal capsular polysaccharide antigens. Restoration of the early IgM response after vaccination is not the most surprising, as TI-2 antigens that enter the bloodstream will normally localise in the spleen followed by a rapid IgM response[21]. The IgG results are concurrent with earlier reports[22] and with the fact that in humans, a single vaccination with Pneumovax® induces significant levels of IgG antibodies. Although the early IgG response might be the consequence of a classic secondary immune response, the non- or low-responsiveness for most PPS subtypes after splenectomy does indicate that this is not likely. In previous studies we have demonstrated that localisation of PPS on follicular dendritic cells in follicle and germinal centre is dependent on a serum factor, most likely a complement-fragment (probably C3d), but not on the presence of specific antibody[16,23,24]. This implies that PPS after entering the marginal zone, may induce PPS-specific B-cells migrating to the follicle/germinal centre[16,25,26], where PPS-complement complexes at the same moment are already localised on FDC[16,23,24]. The germinal centre is considered to be the area where the isotype-switch takes place, enabled by immune complexes on local follicular dendritic cells. Tentatively, it can be suggested that specific for this type of antigen an early isotype switch to IgG can take place independently of the presence of substantial levels of specific IgM.

It should be noted that the clinical presentation of a pneumococcal infection in splenectomized patients (i.e. pneumococcal sepsis) is unlike that of other patients with increased susceptibility to pneumococcal infections due to insufficient anti-polysaccharide antibodies. In the latter category of patients, pneumococcal infections may cause pneumonia, meningitis or otitis, but seldom results in sepsis. This can be explained, because when a functional spleen is present, even badly opsonized pneumococci can be cleared by the spleen MPS, due to the specific low flow spleen red pulp architecture[21] thus preventing bacteremia and sepsis. As in the case of splenectomy the restoration of

clearance by autotransplantation is insufficient[11], and restoration of the humoral response becomes even more important, allowing good opsonization of PPS.

Despite the observation of normal total IgM or IgG antibody titres against TI-2 antigens, a low or absent response to individual subtypes may be present. The capacity for an early, adequate humoral response is of utmost importance, as in case of pneumococcal bacteremia with risk of sepsis, only protection can be expected when a rapid humoral response can be elicited. Only in this case the bacteria may be opsonized. When small amounts of regenerated autotransplanted spleen are present, with adequate response capacities to TI-2 antigen, specific antibodies will be formed. These antibodies are able to cause opsonization of the antigen (encapsulated bacteria) and, because phagocytosing capacity of the splenic fragments will not play an important role, the cells of the mononuclear phagocyte system as present in the liver are then capable of removing these opsonized particles[21].

The architecture of the splenic transplants demonstrated that we were dealing with regenerated splenic fragments with a largely restored white pulp. A functional white pulp is characterised by follicles with a functional germinal centre and a distinct marginal zone that is occupied with B lymphocytes, capable of inducing a primary immune response against TI-2 antigens in an appropriate vascular micro-environment. From the extensive studies by Pabst and co-workers, it was concluded that the younger the recipient and the donor, the better the regeneration and perfusion of the regenerated splenic tissue[27,28]. In addition to these results which showed mainly adequate regeneration when using fetal/newborn spleens, we obtained similar results using rats of 3 months old (”adolescent”). This may be due to strain differences. Although for the human situation an estimation of age range, probably limiting the success of spleen autotransplantation, is hard to give, it seems likely that there is a much better potential for splenic regeneration in children and possibly young adults, than in older subjects.

The question how much spleen tissue should be transplanted has been a matter of controversy with respect to both total amount of transplanted tissue and size of the individual spleen fragments[9,19]. In different animal models the implanted amount of spleen tissue did not affect the resulting amount of regenerated tissue, whereas a particle size does seem to play a role[27]. Others and we think that particle size should at least allow the reticular/stromal structures of individual white pulp nodules to remain intact[27] and should allow ingrowth of proliferating vessels at the implantation site. Although a minimum amount of transplanted spleen tissue seems necessary, a too large mass will result in increase of necrosis, resulting in fever and other complications.

In our study the response capacity was evaluated 12 weeks after transplantation, as for rats it was reported[27] that regeneration of spleen transplants was in general completed. For other species it may take more time to complete this regeneration; 3-6 months is supposed to be the maximum time frame in which regeneration is completed. At present it is not known whether at this time point also functional maturation is reached, although from our results it appears that at least an initial capability to respond to TI-2 antigens is present. Future studies should reveal whether improvement of immune responses could be

obtained when postponing the antigen challenge.
The conclusion of this study is that autotransplanted splenic tissue in the omentum major after splenectomy has the possibility to grow out to immunological functioning spleens with a functional marginal zone. Consequent on this we demonstrated the capacity of early specific antibody formation in the autotransplanted rats comparable to normal, whereas after splenectomy a significantly decreased response was observed for most PPS-types. Autotransplantation followed by immunisation after a time interval will lead to significant increase of antibody levels for most PPS types in most subjects comparable to sham operated rats, whereas after splenectomy a significantly lower number of subjects show a relevant increase in antibody levels. In particular because high immunoglobulin levels are already found at an early time point (3 days) this may give protection against OPSI and offer a survival benefit. Although this has also to be proven in humans, these findings are a positive argument that autotransplantation of splenic tissue is of value and may provide protection against OPSI.

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Chapter 6

The utility of the accessory spleen: a spare part after (accidental) splenectomy

Leemans R*, Harms G‡, Timens W‡
*Dept. Surgery, Medical Centre Leeuwarden and ‡ Dept. Pathology, University Hospital of Groningen, The Netherlands.
Submitted.

Abstract

Splenectomy causes loss of splenic function. Loss of this function results in a compensated loss of filter and phagocytic function and a life-long increased risk of infection or sepsis caused by bacteria with polysaccharide capsules. The only opportunity to prevent this increased risk is to restore or save residual functional splenic tissue. This may be achieved by the surgical preservation of accessory spleens, provided that these can perform normal splenic immunological functions.
Accessory spleens were examined from 10 patients and compared with normal spleens. The histological immunohistological structure of the accessory spleens was similar to that of the normal spleens. This included the capacity to bind pneumococcal polysaccharides. This suggests that accessory spleens may be able to replace normal splenic immunological function, including its key ability to mount antibody responses to encapsulated bacteria. Consequently, splenectomy for reasons other than diseases such as idiopathic thrombocytopenic purpura (where residual splenic tissue may cause recurrence of disease) should not include the removal of accessory spleens, so preservation of these structures could be a valuable and easy way to prevent infection and sepsis after splenectomy.

Introduction

Accessory spleens or splenunculi are well known in surgery. Generally, they are considered to represent congenital ectopic splenic tissue. The frequency of occurrence of accessory spleens is not exactly known, but it is estimated to be between 10 and 30%. A decrease in frequency is described in the first decades of life. This seems due to a process of atrophy of the accessory spleens, which resembles the involution of organs in older age and is considered to be physiologic. The localization varies widely, but the most common locations are as follows (in descending order of frequency): hilus and vascular pedicle of the spleen, around the tail of the pancreas, greater omentum along the greater curvature of the stomach, mesentery of the small and the large intestine, near the left ovary or left testis and in the pouch of Douglas (*fig. 1*).
Most often, there is only one accessory spleen (85%), sometimes two (14%) and rarely three or more (1%).
The etiology of the development of accessory spleens is unknown. Some authors state that it results from the embryological development of splenic tissue with imperfect fusion of the separate splenic masses. Others attribute them to intra-uterine trauma in the course of fetal life. Accessory spleens are often seen in combination with other congenital anomalies.
Accessory spleens have traditionally been considered superfluous, without significant immune function. Their histology is variously described as being similar to normal spleens or as being imperfect. In general, accessory spleens are considered to be expendable.
Splenectomy or functional asplenia causes loss of splenic function. Several of these functions, such as the filter function and phagocytic function, are compensated for over

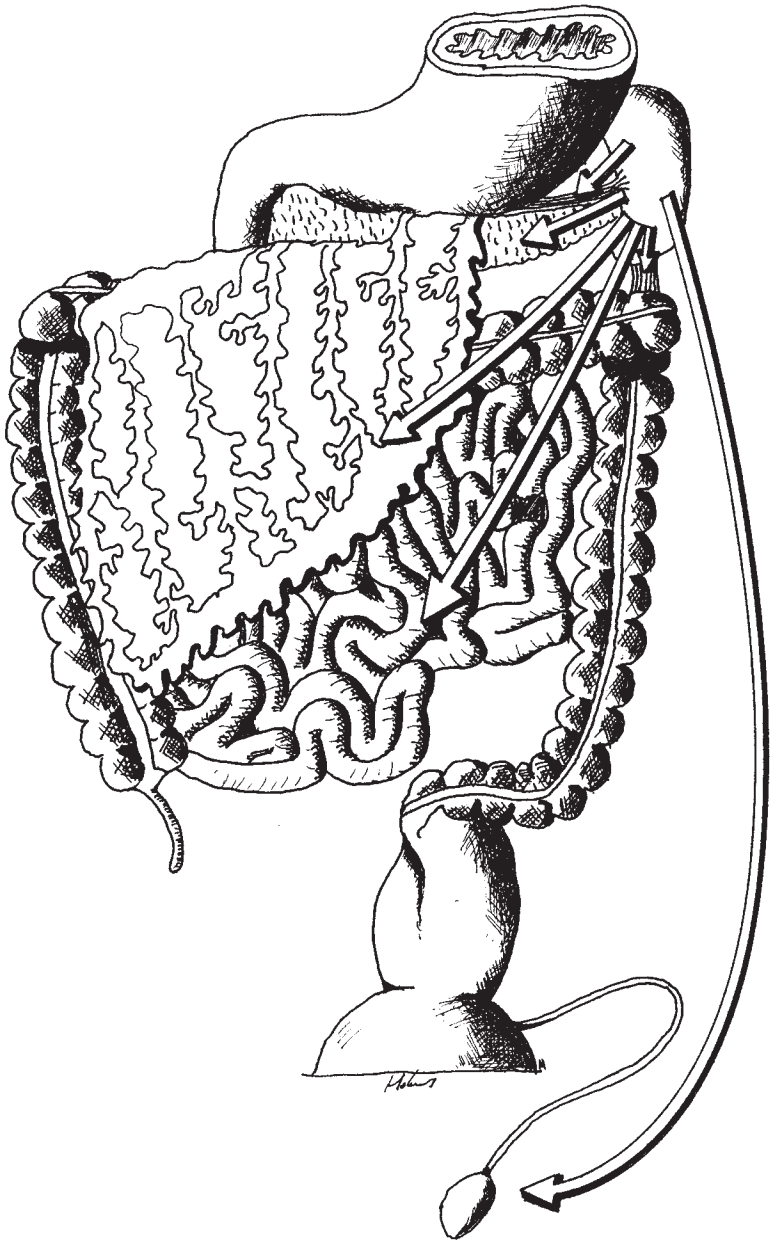

Fig.1. Most common locations of accessory spleens.

time. A specific immune function, which is limited to the spleen, is the induction of primary humoral immune responses to encapsulated bacteria such as S. pneumoniae. Consequently, splenectomy results in loss of this function with a life-long increased risk of infection or sepsis caused by bacteria having polysaccharide capsules. The only opportunity to prevent this increased risk is by restoring or saving residual functional splenic tissue. This may be achieved by the surgical preservation of accessory spleens, provided that these can perform all normal splenic immune functions. In previous studies we have demonstrated that pneumococcal polysaccharides localize in the splenic marginal zones and germinal centers. In the present study, we have evaluated whether these functionally important compartments are present in accessory spleens and whether these cellular compartments have the capacity to bind pneumococcal polysaccharides.

Patients and methods

Accessory spleens were excised from 10 patients undergoing a laparotomy for other reasons (gastric and colonic cancer, abdominal trauma). As controls, spleens were obtained from trauma patients after splenic injury (n=10). In 4 additional cases, tissue from spleen as well as accessory spleen was available from the same patient (all non-involved spleens in operations for gastric cancer).

Histology

Part of the splenic tissue was frozen at -80°C in liquid isopentane and stored in a freezer at the same temperature. Other parts were fixed in formalin and embedded in paraffin. For histological examination, 3µ sections were cut and stained with hematoxylin-eosin.

Antigens

For immunohistological localization studies PPS types 1, 4, 6A, 9N, 9V, 10A, 12F, 14, 19A and 19F (American Type Culture Collection, Rockville, Maryland, USA) were diluted in distilled water, to a final concentration of 1 mg/ml.

Antibodies

Peroxidase-conjugated rabbit anti-mouse immunoglobulin antiserum (RAM^{per}) and peroxidase-conjugated swine anti-rabbit immunoglobulin antiserum (SAR^{per}) were obtained from Dakopatts (Glostrup, Denmark). To detect the different types of PPS antigens, type or group specific rabbit anti-pneumococcal polysaccharide immunoglobulins (anti-PPS) were used (State Serum Institute, Copenhagen, Denmark).
Anti-CD2 (Leu-5b) and anti-CD3 (Leu-4) monoclonal antibodies (mAb) were used to detect all T-cells. Anti-CD20 (L26) and anti-CD22 (Leu-14) mAb were used to detect B-cells. Anti-CD21 (HB5) is a specific antibody for the C3d/EBV receptor (CR2).

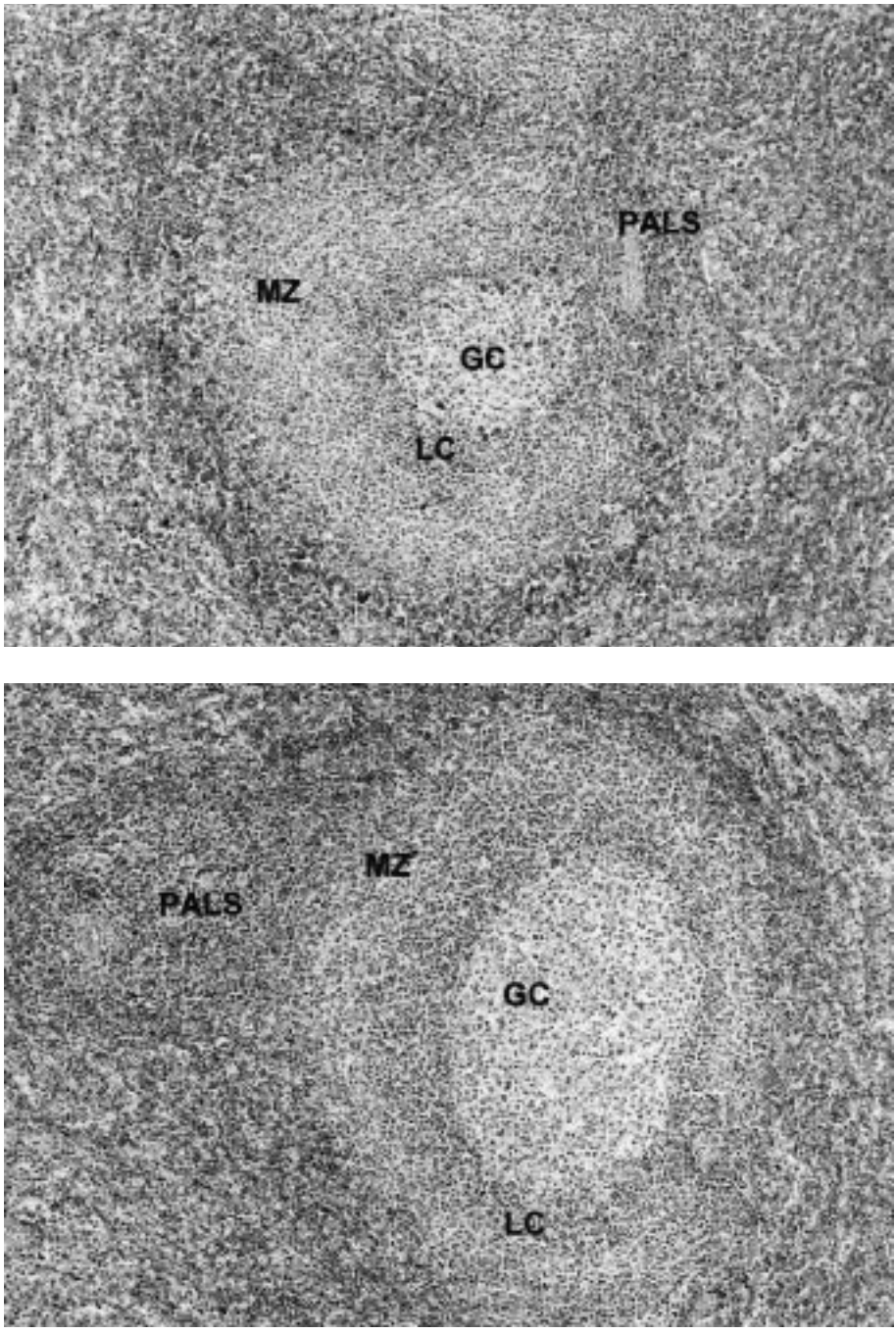

Fig.2. Histology of an accessory spleen (a) and of a normal spleen (b) of the same patient (hematoxylin eosin, x 150).

L26 (CD20) and anti-CD35 (CR1) detecting the C3b receptor were obtained from DAKO (Glostrup Denmark). All other antibodies were from Becton-Dickinson (San Jose, CA, USA). An immunoperoxidase staining procedure was applied as follows. Five micron frozen sections, air-dried for 20 min and fixed in acetone (100%) for 10 min at 20°C, were incubated for 30 min with the different Mab at optimal dilutions, as determined separately. Next, sections were incubated with RAM^{per} diluted 1:20 in PBS with 5% human AB serum for 15 min. Every incubation step was followed by rinsing the sections with phosphate buffered saline (PBS) during 5 min with 3 changes. The peroxidase activity was visualized using 3-amino-9-ethylcarbazole (AEC) and H_2O_2. Finally sections were washed in distilled water and counterstained in Mayer's hematoxylin, rinsed in running tap water and covered with Kaiser's glycerin-gelatin and a cover slip.

Detection of PPS-binding

Cryostat sections (5 µm) of the human spleens were picked up on glass slides and kept at -20°C until use. The different PPS-types were preincubated for 1 hour at 37°C, in a dilution of 1:4 (0.25mg/ml) in normal human serum (NHS) respectively. An immuno-peroxidase procedure was performed similar to that described above using SAR^{per} instead of RAM^{per}, for the detection of the rabbit antisera. Sections were air-dried for 20 min and fixed in acetone (100%) for 10 min at 20°C and were then incubated for 30 min at 37°C with the different types of PPS. This was followed by incubation for 30 min with the different type or group specific rabbit antisera (State Serum Institute, Copenhagen, Denmark) anti-PPS 1, 2, 3, 4, 6, 9, 10, 12, 14 and 19 respectively, which were used at optimal dilutions, as determined separately, with 5% human AB serum (heat-inactivated). As controls for the immunohistological staining procedure, sections were used in which the incubation with PPS or the incubation with PPS-specific antiserum was omitted or the type-specific anti-PPS serum was replaced by non-relevant rabbit antisera against human factor VIII or CD3 (both DAKO). Also, sections were incubated with PPS without preincubation with normal human serum.

Results

The accessory spleens were found near the hilus of the normal spleen and in the greater omentum, and had a normal appearance. The weight ranged from 20 to 100 grams.
Histological examination of the accessory spleens showed normal splenic structure with normal red and white pulp in all cases (*see also fig. 2 page 107*). Primary and secondary lymphoid follicles with germinal centers were present. Significantly, also an anatomically normal marginal zone was found.

Immunohistochemistry

Splenic white pulp immuno-architecture was completely similar to normal spleen.

Staining results were consistent and reproducible. Staining patterns obtained with PPS-types were compared with CD2/3, CD20/22, CD21 and CD35 stained sections. CD2/3 and CD20/22 were used to delineate T- and B-cell compartments. These showed the expected configuration in all spleens. CD21 and CD35 show staining of follicular dendritic cells and B-cells from the mantle zone (CD21 rather weakly) and marginal zone. CD35 also reacts with granulocytes and more weakly with histiocytes/monocytes.
Sections that were stained using NHS-incubated PPS during the procedure revealed a common staining pattern for all types of PPS for all adult spleens of non-vaccinated patients (*fig. 3*). PPS-binding was seen in germinal centers (GC's), mantle zones and marginal zones. GC's showed a strongly stained meshwork pattern, identical to the CD21 and CD35 pattern as demonstrated previously. The periarteriolar lymphocyte sheath (PALS) and the red pulp were negative.

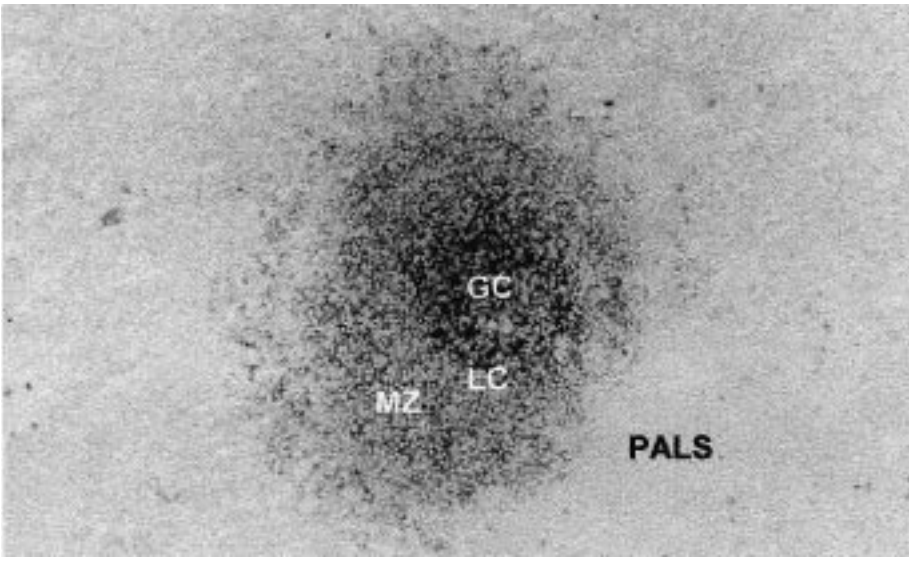

Fig. 3. Localization of PPS type 4 on marginal zone B-cells and follicular dendritic cells of an accessory spleen supporting normal immune function (immunoperoxidase, x 150)

Discussion

Historically, accessory spleens were considered to be functionally unimportant and were therefore often removed during laparotomy. In hematological diseases such as idiopathic thrombocytopenic purpura (ITP) it is known that accessory spleens can be the cause of recurrence of the disease after splenectomy, as they take over part of the normal functions (mostly storage and phagocytosis) of the removed spleen. In this situation there is a valid clinical indication to remove all splenic tissue that is present.

Accessory spleens as well as seeded traumatic spleen fragments (splenosis) has been reported to take over at least part of the function. There are even cases recorded after splenectomy for trauma in which an unremoved accessory spleen has grown as large as a normal spleen.
These facts, together with the normal histology of the accessory spleens found in this study, suggest the possibility of a normal immune function.
In our study we were able to confirm the normal histologic morphology of accessory spleens as compared to the orthotopic spleen. More importantly, immunohistochemistry showed that the immuno-architecture of accessory spleens was remarkably similar to that of the normal, orthotopic spleen. Marginal zones were present including high expression of CD21, characteristic of mature humoral immune function. Germinal centers were present in many, but not all accessory spleens, as would be expected in humans. Localization of different types of pneumococcal polysaccharides was observed on marginal zone B-cells and follicular dendritic cells similar to that found in the normal adult human spleen. This suggests that accessory spleens may not only have a normal phagocytic and storage function but may have a normal immunological function as well. If this is the case, accessory spleens may be expected to play a role in the primary immune defense, especially to T-cell independent antigens type 2 (TI-2 antigens) such as PPS.
After resection of the orthotopic spleen the primary immune defense to TI-2 antigens is diminished. This results in an increased risk for the overwhelming post splenectomy infection syndrome (OPSI). If accessory spleens can indeed have an immunological function comparable to the normal spleen, this serious complication may be diminished in the presence of accessory spleens after vaccination against TI-2 antigens, such as Streptococcus pneumoniae (e.g. Pneumovax®). Further studies examining immune responses in the presence of absence of accessory spleens need to be carried out in order to confirm this hypothesis.
In conclusion, our results demonstrate that, following removal of an orthotopic spleen, accessory spleens are able to take over not only storage and phagocytic functions, but also show histological and immunohistological features suggestive of normal immunological function. As the induction of the humoral response to TI-2 antigens such as encapsulated bacteria is completely dependent on the spleen and cannot be effected by other lymphoid tissues, we recommend to leave accessory spleens in situ when there is no specific indication for removal.

Acknowledgements

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Chapter 7

SUMMARY, DISCUSSION AND CONCLUSIONS

Summary, discussion and conclusions

In the introduction of this thesis it has already been pointed out that the spleen is more important than initially was thought. The spleen has an important role in the defence system of the body against blood-born antigens, and is essential in blood clearance of poorly opsonized antigens. Consequently the spleen is capable of the clearance of thymus independent type 2 (TI-2) antigens, such as polysaccharide encapsulated bacteria like Streptococcus Pneumoniae, which are generally poorly opsonized. The spleen has an important role in the primary and secondary antigen-specific immune response to blood born antigens. After a splenectomy changes in the immunological defence system are known and this can result in the overwhelming post splenectomy infection (OPSI) syndrome, especially in children, with a high mortality.
It is therefore important to preserve the immune function of the spleen. The best way to do this is by preserving the spleen itself or part of it, or alternatively, if this is not possible, by preserving some splenic tissue, providing an alternative blood supply. The latter is the aim of spleen autotransplantation after splenic trauma.

In chapter 1 an overview is given of the historical background of the role of the human spleen, the role of the spleen in the immune system, the consequences of splenectomy, and the aims of this thesis.

In chapter 2 a description is given of a technique of wrapping an absorbable net around a ruptured spleen. In a study of 31 patients with severe traumatic rupture of the spleen good results were achieved by this technique. However, it is difficult to operate on the spleen in an emergency situation and it is also difficult to decide which therapy is the best in the given situation. The general opinion at present is that this operation should be viewed as a speciality manoeuvre that requires considerable experience. In the addendum of this thesis an algorithm is given. One has to keep in mind that the risks of the procedures used for preserving the spleen should not exceed the overall risks of a splenectomy.

In chapter 3 the mononuclear phagocyte system (MPS) function was studied using the Fc-receptor test. In most studies after an autotransplantation of splenic tissue (AST) where a scintigram was made, ^{99m}Tc colloid scans were used to evaluate the MPS function. We used, ^{99m}Tc-labeled rhesus-positive erythrocytes coated at low density with anti-rhesus antibody. This test is considered to provide a parameter for splenic MPS Fc-receptor function, allowing capture of low-opsonized particles/antigens. This was done in 24 patients who underwent splenectomy, 10 of whom underwent splenic autotransplantation. All patients undergoing AST showed a hot spot at the site of splenic implantation indicating the presence of Fc-receptor-bearing (phagocyte) cells. In 8 of the non-transplanted patients a hot spot was also found and considered to be ectopic splenic tissue. The kinetic of the Fc-receptor test is normally bi-exponential, but in this study it showed a delayed and monoexponential blood clearance in all patients. There were no significant

differences between the patient groups. The results of the analyses of the kinetics of the Fc-receptor-bearing cells were not characteristic of adequate restoration of the overall splenic Fc-receptor function. Based on this study, autotransplantation of a small amount of splenic tissue after splenectomy cannot be considered sufficient to restore splenic MPS function, especially in relation to low-opsonized antigens and is inadequate for blood clearance. To achieve this effect an adequate blood flow through the autotransplant is needed as well as a rather large volume of tissue.

In chapter 4 we studied the restoration of the humoral immune response to pneumococcal capsular polysaccharides and the phagocytic function of granulocytes in 10 human subjects undergoing autologous spleen transplantation (AST) after splenectomy compared to 14 subjects after splenectomy only. The phagocyte activity showed normal results in relation to the reference values, and no significant differences between the patient groups. To evaluate the antibody responses we measured the cell wall polysaccharide antigens without distinguishing between antibodies directed against capsular polysaccharides or cell wall polysaccharide antigens. A specific ELISA was used with an absorption step to remove antibodies against cell wall polysaccharide antigens. We used capsular antigens and this showed a surprising effect of AST after splenectomy on specific antibody responses after pneumococcal vaccination.
Significant antibody titre rises were found for both IgM and IgG in the AST patients as compared with the patients without splenic regrowth. A partial improvement was seen in patients with splenosis (accidental seeding of splenic tissue in the abdominal cavity) and no rise was seen in the non-transplanted group without splenosis. Considering this significant antipneumococcal antibody increase, after splenectomy AST can be expected to improve the specific humoral response to pneumococcal infections and presumably also to other TI-2 antigens. This study showed also that there is a strong possibility that ”born-again” or accessory spleens will provide immune protection after splenectomy. However, failure of ectopic splenic tissue to prevent OPSI after splenectomy has been described.
Although it is not yet clear whether complete protection against all pneumococcal subtypes can be obtained, AST may be expected to help limit the risk of the OPSI syndrome. This demonstrates that AST can play a role in the management of severe splenic injury in which splenectomy is inevitable, particularly when, at an appropriate time point, followed by vaccination with polyvalent pneumococcal vaccine.

In chapter 5 the evaluation is described of the restoration of the humoral immune response after splenic autotransplantation in a rat model. The rats were divided into 3 groups; splenectomy, splenectomy with AST and sham operation, and all vaccinated with 23-valent pneumococcal vaccine. In this study the type specific anti pneumococcal polysaccharide ELISA with absorption of antibodies to cell wall polysaccharide antigens was used. The results of this study support our findings in humans as described in chapter 4. Significant antibody titre rises of IgM and IgG were found in the autotransplanted rats,

comparable to sham-operated rats for most types without significant differences. Splenectomized rats showed significantly lower increase in Ig-levels. The titres were highest 3 days after vaccination.
The architecture of the autotransplants demonstrated fully regenerated splenic fragments with a normal white pulp. Immunohistochemical studies demonstrated structurally functional autotransplants including an intact marginal zone with B lymphocytes and macrophages. It appears that, with respect to both architecture and immune capacity, the autotransplants are capable of inducing a primary immune response against TI-2 antigens. This study showed that even small amounts of regenerated splenic autotransplants can provide an adequate humoral response to several pneumococcal capsular polysaccharides, and can be expected to give improved protection against OPSI, especially in combination with vaccination.
From our and other animal studies it should be concluded that to obtain the best results of vaccination after spleen autotransplantation, the vaccination should be given at a time-point at which complete splenic regrowth can be expected. Keeping the limitations of an animal model (including strain and species differences) in mind, it should be advised to give the vaccination not earlier than three months, but most likely better at 6 months after autotransplantation.

In chapter 6 the examination of accessory spleens of 10 patients is described and compared with normal spleens. The histological as well as the functional (immuno-histological) structure of the accessory spleens was found similar to that of the normal spleens. This includes the capacity to bind pneumococcal polysaccharides. These findings, in combination with our findings reported in chapter 2 to 4, imply that after removal of the orthotopic spleen, accessory spleens can be expected to take over the most important humoral immune function. This includes the spleen specific ability to initiate a primary humoral response to encapsulated bacteria like pneumococs. Consequently preservation of accessory spleens would be a valuable way of preventing infection and sepsis after splenectomy because of trauma.

Conclusions

The following conclusions can be drawn from this thesis:

1 A severely injured spleen can often be saved by wrapping in an absorbable net. This operation should be considered a speciality manoeuvre that requires considerable experience.
2 Spleen autotransplantation after splenectomy provides some mononuclear phagocyte system activity but is inadequate for blood clearance.
3 The spleen has an important and unique role in the initiation of the specific immune response, especially to the T-cell independent type 2 antigens such as pneumococs.

4 Autotransplanted splenic tissue is capable of at least partial restoration of specific anti-pneumococcal humoral immune function after splenectomy.

5 Spleen autotransplants have a normal white pulp lymphoid compartment, including a functionally intact marginal zone.

6 Autotransplantation of splenic tissue after splenectomy, especially in combination with (pneumococcal) vaccination, can be expected to improve protection against the OPSI syndrome.

7 The functional anatomical compartments of accessory spleens do not differ from normal spleens and therefore can be expected to perform normal splenic immune functions. Consequently, when accessory spleens are encountered during routine surgical procedures, not involving spleen pathology, it is strongly advised to leave them in situ.

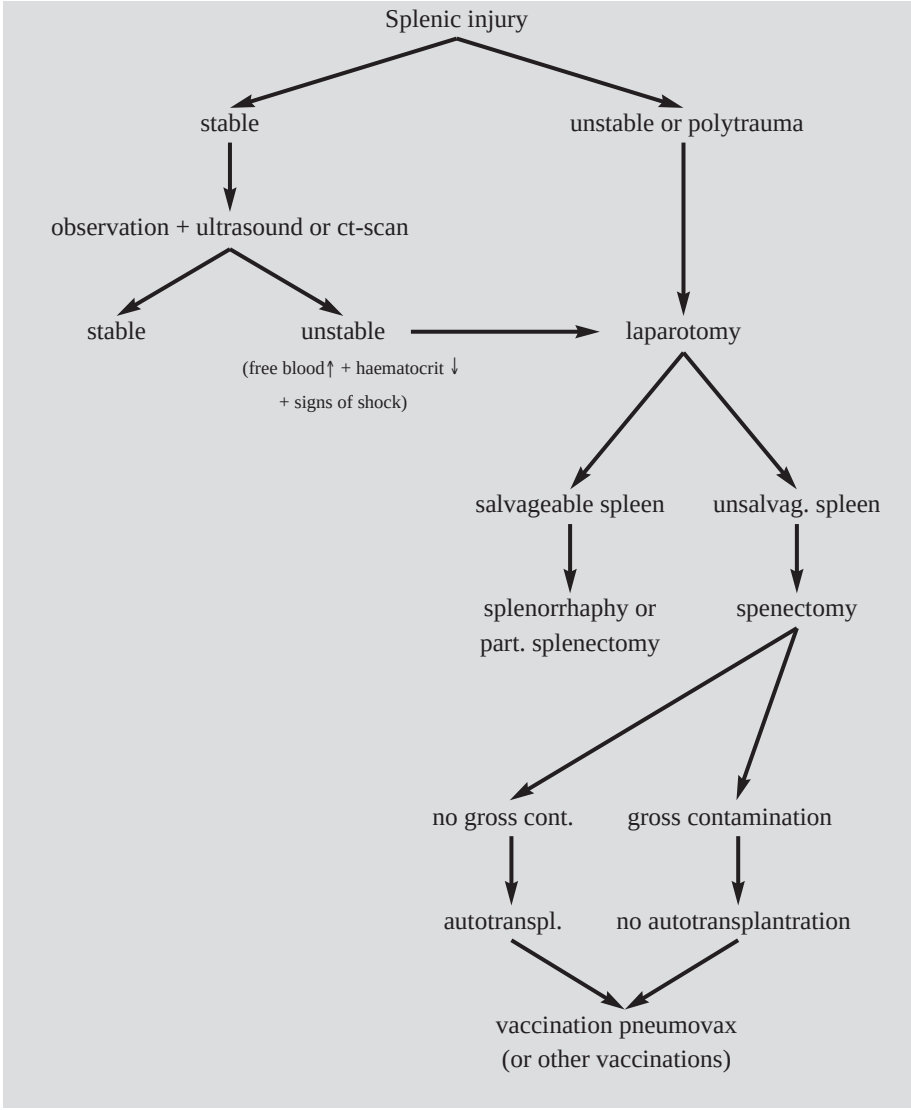
Perspectives

Splenic trauma does not occur very frequently. Therefore, a patient with splenic trauma ideally should be admitted to a hospital with a surgical department with special attention for or specialised in trauma surgery and sufficient knowledge of and experience with spleen salvaging techniques, such as use of an absorbable mesh. Trauma surgeons should realise that the outcome of use of the mesh-wrapping technique is dependent on their particular attention to the procedure[1,2].

The steps to perform after a patient with a rupture of the spleen has been admitted to hospital are shown in a flow-chart in table I. In short the following steps are advised[3,4,5]. The first priority has to be to save the spleen. This can be tried without an operation when the patient is clinically stable (stable vital functions without signs or threats of splenic bleeding). In case of an unstable patient a laparotomy has to be performed. When considered feasible, attempts should be made to wrap the spleen in an absorbable net. When a splenectomy is needed, autotransplantation of splenic tissue should be performed in the omentum, as in this way vascular supply and ingrowth of vessels is most easily enabled. Autotransplanted spleen tissue should not be put through a mesh, but preferably should be cut in particles of 5 to 10 mm^3 to preserve microarchitecture. This intact splenic microarchitecture seems essential for repopulation and restoration of specific splenic lymphoid compartments[6]. Although the ideal amount of tissue to be transplanted is unclear, most likely this should be at least 20-50 gram.

After autotransplantation, anti pneumococcal vaccination is advised with a time interval of at least three months, or when feasible six months. As this time interval between auto-transplantation and vaccination still is deduced from animal experiments[6], future prospective human studies, with an increased number of patients, should provide information about the optimal vaccination time point.

Table I Flow chart for splenic injuries. According to Witte, Esser and Rapaport[3]



The use of a batch of specific spleen-dependent antigens, such as different pneumococcal polysaccharide subtypes, can provide a procedure for testing functional splenic immune response capacity in man[7]. This can be done after spleen autotransplantation, but also after other spleen operations or in case of other spleen pathology. In such a ”test-vaccine” it is important to include a sufficiently wide range of antigenic variety of epitopes.
Within an individual patient, the use of a test-vaccine could provide information about the success of the spleen saving procedure. In addition this would provide information about

humoral response failures to specific antigens in the vaccine. The latter is of importance as in this way information is obtained that this patient likely is not able to respond properly to one or more specific subtypes of microbial species. In this case, when such a patient would be admitted to the hospital with a possible diagnosis of infection, serology may not provide adequate information, as the patient apparently was not capable to rise a humoral response to some antigens! In such case the diagnostic approach should be directed to direct detection of the (microbial) antigen.
When, by using the test-vaccine, insufficient protection by the spleen-saving procedure or partial humoral response failure to some antigens is demonstrated, the use of protein-conjugate vaccines should be considered. Vaccines consisting of a protein (like tetanus toxoid) conjugated to bacterial polysaccharides can be expected to be able to initiate an adequate response to the polysaccharide, without the need for presence of a functional spleen[8,9,10]. Despite this the use of protein-conjugate vaccines should not be considered a replacement for spleen preserving procedures. It is clear that spleen preserving procedures like spleen autotransplantation have the advantage that potentially the spleen-specific humoral response capacity to theoretically any encountered TI-2 antigen would be restored, whereas the use of conjugate vaccines only gives protection to the antigens used in the vaccine.

Whereas the role of the spleen in the immune system has been recognized in recent years, it was not readily clear whether preservation of the spleen in case of trauma would add anything to outcome with respect to morbidity and mortality. With the results presented in this thesis the importance of spleen preservation has been supported. The spleen auto-transplantation studies have shown that this procedure significantly improved the immune response capacity against relevant bacteria, related to postsplenectomy morbidity.
Future studies should aim at further improving the effects of autotransplantation by optimizing the outgrowth of the amount of spleen tissue and determination of optimal vaccination time point.
When splenectomy is unavoidable in a patient with a splenic trauma, spleen auto-transplantation combined with anti pneumococcal vaccination should be considered a valid option to reduce the risk of subsequent morbidity.

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Chapter 8

MET ANDERE WOORDEN

Inleiding

Gedurende vele eeuwen zag men de milt als een mysterieus orgaan. Erasistratus geloofde dat de milt er was voor de goede symmetrie in de buik en Plato geloofde dat de milt de lever in een goede conditie hield. Volgens Hippocrates zorgde de milt voor een goede balans tussen de vier essentiële lichaamssappen in het lichaam; namelijk bloed, slijm, gouden gal en zwarte gal.
Pas in de 17e en de 18e eeuw werd goed anatomisch onderzoek van de milt uitgevoerd en werden ook de eerste functies van de milt ontdekt. Dat de milt een functie heeft in de afweer werd pas in het begin van de 20e eeuw ontdekt. Het duurde echter nog tot de publicatie van King en Shumacker in 1952 voordat men veronderstelde dat er een toename zou kunnen zijn van infecties na het verwijderen van de milt. Pas in de tijd hierna werd er serieus onderzoek gedaan naar de afweerfuncties van de milt.
De milt is een orgaan van ongeveer vuistgrootte dat ligt onder de linker ribbenboog. Het heeft een zeer goede bloedtoevoer, ongeveer 25% van het bloed dat het hart uitpompt passeert de milt. In de milt zelf is een systeem aanwezig, dat er voor zorgt dat een aanzienlijk deel van dit bloed heel langzaam gaat stromen. Op deze manier ontstaat er een heel goed contact tussen het passerende bloed en de cellen van de milt. De milt kan op deze wijze gemakkelijk constateren dat er bepaalde stoffen (antigenen) in het bloed zitten die er niet horen. De milt reageert hier onmiddellijk op door het maken van antistoffen of antilichamen. Deze antistoffen gaan dan als een soort vlaggetjes op de betreffende antigenen zitten waardoor elders in het lichaam geconstateerd kan worden dat deze niet in het lichaam thuis horen. De z.g.n. opruimers (fagocyten of macrofagen) kunnen aan de hand van deze vlaggetjes de betreffende antigenen herkennen en verwijderen. Dit fenomeen, dat de humorale afweer genoemd wordt (in tegenstelling tot de cellulaire afweer), is één van de belangrijkste functies van de milt. Daarnaast zijn er ook nog andere functies, zoals het weg filteren van beschadigde rode bloedcellen, het vormen van een reservoir voor witte bloedcellen en bloedplaatjes, en zorgen voor de productie van tuftsine, een stof die de activiteit van de opruimcellen (fagocytose) bevordert.
Het verwijderen van de milt heeft een aantal gevolgen. De belangrijkste is de vermindering van de afweer tegen met name bacteriën die een omhulsel van suikermoleculen hebben, zoals de pneumococ. Het gevolg hiervan is dat er een verhoogde kans (3-5%) bestaat op ernstige bloedvergiftiging (sepsis) met dit soort bacteriën, dat in de Engelse literatuur bekend staat als: Overwhelming Post Splenectomy Infection (OPSI). Er is een grote kans om hier aan te overlijden, met name bij kinderen (50%).
Dit heeft er toe geleid dat men technieken is gaan bedenken om de milt bij beschadiging na een ongeval te kunnen behouden. Dit wordt verder in hoofdstuk 2 uitgewerkt.
Het is gebleken dat miltcellen die zijn verdwaald in de buik als gevolg van een miltletsel kunnen gaan uitgroeien tot nieuwe kleine miltjes (splenose genoemd) en dat deze een functie zouden kunnen hebben in de afweer wanneer de eigenlijke milt is verwijderd. De volgende stap was het bewust terug plaatsen van stukjes milt, de autotransplantatie. Onderzoek hiernaar wordt verder behandeld in de hoofdstukken 3 t/m 5.

Bij vrij veel mensen komt als een soort anatomische variant voor dat al bij de geboorte extra miltjes in de buik aanwezig zijn; de zgn. bijmilten. Het is gebleken dat deze bijmilten een normale weefsel opbouw hebben en dus mogelijk een rol kunnen spelen in de afweer, wanneer de oorspronkelijke milt is verwijderd. Dit wordt besproken in hoofdstuk 6.
De belangrijkste aanleiding voor het starten van het promotieonderzoek was de vraag of de autotransplantaties van stukjes milt, na een miltverwijdering i.v.m. een ongeval, een afweerfunctie hebben. Met andere woorden of het zinvol is om een autotransplantatie uit te voeren.

Hoofdstuk 2. Een miltsparende techniek met een resorbeerbaar net

De behandeling van miltrupturen is het onderwerp geweest van uitgebreide discussies. In het verleden werd de verwijdering van de milt gezien als de beste behandeling totdat het duidelijk werd dat hierdoor een verhoogde kans ontstond op een ernstige bloedvergiftiging. In onderzoek bij dieren en later ook in klinische studies bij mensen werd beschreven dat sommige geruptureerde milten behouden konden blijven door deze in een resorbeerbaar (oplosbaar) net te wikkelen. In hoofdstuk 2 worden 31 patiënten beschreven met een geruptureerde milt die zijn behandeld met een miltsparende methode door gebruikmaking van zo'n resorbeerbaar net. Bij 30 patiënten van deze groep waren geen tekenen van bloedingen. Eén patiënt kreeg een vergroeiing in de buik met verstopping van de darmen. Deze moest daaraan worden geopereerd en kreeg na de operatie een infectie van de wond. Bij één patiënt werden 2 netten over elkaar gebruikt wegens een uitgebreide verscheuring van de milt. Dit resulteerde in het afsterven van deze milt, waarbij de milt zelf echter niet geïnfecteerd raakte. In totaal waren er dus slechts 2 milt gerelateerde complicaties bij de 31 patiënten als gevolg van deze behandeling.
Op basis van deze resultaten werd gesteld dat een miltsparende behandeling de eerste optie moet zijn bij een miltruptuur ten gevolge van een ongeval. Het resorbeerbare net heeft bewezen daarin een belangrijke aanwinst te zijn.
De conclusie is dat het resorbeerbare net veilig is, maar dat ervaring vereist is bij deze niet eenvoudige operatietechniek. Gezien de goede resultaten, wanneer toegepast in een adequate setting, heeft deze techniek een belangrijke plaats verworven in de miltsparende behandeling bij ongevalletsels van de milt.

Hoofdstuk 3. Fc-receptor functie na miltautotransplantatie bij de mens

De Fc-receptor test is een test waarbij gemeten wordt of bepaalde cellen van het fagocyten systeem lichaamsvreemde cellen of stoffen uit het bloed kunnen halen die een relatief geringe bekleding hebben met antistoffen. Juist het fagocytensysteem in de milt is in staat om dit te doen door de specifiek lage bloedstroomsnelheid in de milt.
Voor de test werden rode bloedcellen genomen die op een bepaalde manier werden bewerkt zodat zij een geringe belading met antistoffen hadden. Tevens werden ze voorzien van een radioactief isotoop zodat zij d.m.v. een gamma-camera buiten het lichaam opge-

spoord konden worden. Een concentratie van de beladen en gemerkte cellen kan door deze camera gezien worden als een zekere mate van activiteit van het fagocytensysteem. Aan de hand van in serie achter elkaar genomen bloedmonsters kan gemeten worden hoe snel deze cellen weer uit het bloed verwijderd worden en dit kan als een z.g.n. bloedverwijderingscurve uitgezet worden.
Deze Fc-receptor test is uitgevoerd bij 24 patiënten waarbij de milt verwijderd was vanwege een ongeval. Bij 10 patiënten was een autologe transplantatie van miltweefsel uitgevoerd van ± 25 gram in het grote vetschort dat voor de darmen hangt (omentum majus). Deze patiënten toonden allen activiteit ter plaatse van de transplantaties. Echter bij 8 van de 14 niet-getransplanteerde patiënten werd ook activiteit gevonden. Dit werd geduid als door het ongeval in de buikholte verspreid resterend miltweefsel na miltverwijdering (ectopisch miltweefsel). Dit kan beschouwd worden als een soort ”natuurlijke” autotransplantatie. Bij slechts 6 patiënten werd dus geen activiteit vast gesteld.
De bloedverwijderingscurve toonde een slechte verwijdering van de bewerkte bloedcellen zonder een significant verschil tussen de verschillende patiëntengroepen. Met andere woorden: de autotransplantaten waren niet in staat om een adequate bloedreiniging of filterfunctie uit te oefenen. Gezien het feit dat het slechts kleine stukjes milt betreft, die bovendien buiten de normale bloedstroom zitten, was dit wellicht ook niet te verwachten. Wel kon geconcludeerd worden dat het filtermechanisme (mononucleaire fagocytose activiteit) wel werkte, alhoewel te laag voor een meetbaar effect in het bloed, en dat de autotransplantaten dus niet waren afgestorven (vitaal waren).

Hoofdstuk 4. Immuun-respons-capaciteit na miltautotransplantatie bij de mens: herstel van individuele pneumococcen-subtypes-response na vaccinatie

In dit hoofdstuk werden een aantal immunologische functies onderzocht bij mensen die een verwijdering hebben ondergaan wegens een ongeval. Het betrof dezelfde patiëntenpopulatie als in het vorige hoofdstuk.
Ter beoordeling van de activiteit van de witte bloedcellen (de granulocyten) werden de ”nitro blue tetrazolium test” en de ”phagocytose killing test”uitgevoerd. Dit zijn testen die de mate van activiteit meten van de witte bloedcellen die elementen, die niet in het lichaam thuishoren, opeten (fagocyteren). Deze testen gaven geen afwijkingen te zien, dus ook geen verschillen tussen de mensen met en zonder autotransplantaties. De ”helix pomatia hemocyanine (HPH) test” toont het vermogen om een algemene immunologische afweerreactie te geven met vorming van antistoffen op een niet-specifieke stof (het HPH). Deze test gaf een normaal reactiepatroon bij alle patiënten met een verhoging van de afweerstof IgM na 6 weken. Tevens werden nog de spiegels bepaald van de immunoglobulines, een aantal complementfactoren (C1q, C3, en C3d) en properdin factor B. Ook deze bepalingen toonden geen afwijkingen.
In het bijzonder werd gekeken naar de mate van afweerstoffenaanmaak (humorale immuun response) tegen pneumoccen. Alle patiënten werden uiterlijk 6 maanden na de operatie gevaccineerd met pneumococcenvaccin. Er zijn verschillende typen pneumo-

coccen; in het vaccin zijn de 24 belangrijkste aanwezig. Net voor de vaccinatie, na 3 en na 6 weken werden bloedmonsters afgenomen ter bepaling van de hoeveelheid IgM en IgG antistoffen tegen de 6 typen uit het pneumococcenvaccin. Deze 6 typen zijn zo gekozen dat de vorming van antistoffen hiertegen een goede weerspiegeling is van de reactie tegen het gehele vaccin.
Deze test toonde een verrassend effect van de autotransplantaties op de vorming van de specifieke antistoffen na de vaccinatie. Een significante stijging werd gevonden bij zowel de afweerstofgroepen IgM als IgG bij de patiënten met autotransplantatie ten opzichte van de patiënten zonder miltweefsel. Een gedeeltelijke verbetering werd gezien bij de patiënten met ectopisch miltweefsel.
Uitgaande van deze significante antistofspiegelstijging tegen pneumococcen mag verwacht worden dat milt autotransplantaten in staat zijn om een sterk verbeterde antistof vorming te geven in geval van pneumococceninfectie en waarschijnlijk ook in geval van infecties met andere zwezerik (thymus) onafhankelijke type 2 antigenen (TI-2 antigenen).

Hoofdstuk 5. Miltautotransplantatie geeft herstel van de functionele lymfoïde compartimenten en herstelt de humorale immuunreactie op pneumococcen-polysacchariden-vaccinatie

Om de resultaten uit het vorige hoofdstuk in een meer gecontroleerde opzet te bevestigen en om te kunnen onderzoeken op welke wijze en in hoeverre het miltweefsel zelf een normale structuur laat zien na autotransplantatie, werd een vergelijkbare proefopzet uitgevoerd in een dierexperiment.
Wistar ratten werden verdeeld in 3 groepen die als volgt werden geopereerd: miltextirpatie, miltextirpatie gevolgd door autotransplantatie en een z.g.n. sham operatie (alleen de buik openen en sluiten zonder verdere handelingen). Na 12 weken werden de ratten met pneumococcenvaccin gevaccineerd. Bloedmonsters ter bepaling van de gevormde antistoffen werden afgenomen na 3 dagen, 3 en 6 weken. Er werd gekeken naar IgM en IgG tegen de 6 representatieve typen pneumococcen.
Na beëindiging van het onderzoek werden de autotransplantaten verwijderd en onderzocht. Er werd een significant verhoogde IgM en IgG antistof stijging gevonden bij de groep ratten met autotransplantaties. Ook het aantal ratten in deze groep dat tenminste een verdubbeling van de antistofspiegel had, was significant groter.
Het onderzoek van de autotransplantaten toonde normaal geregenereerd miltweefsel. De autotransplantaten hadden een normale structuur, waarbij ook gespecialiseerde afweercellen op de normale miltspecifieke plaats aanwezig waren.
Dit onderzoek bevestigde dat autologe transplantaties van miltweefsel een adequate afweerstof (humorale) reactie kan geven op verschillende pneumococcen typen en dat verwacht kan worden dat ze een zekere mate van bescherming zullen geven, met name in combinatie met een vaccinatie. Op theoretische gronden lijkt het het beste om deze vaccinatie niet eerder te geven dan na 3-6 maanden, omdat dan de autotransplantaten volledig zijn geregenereerd.

Hoofdstuk 6. Het nut van bijmilten: een reserve onderdeel na een (ongeval gerelateerde) miltextirpatie

Een bijmilt kan omschreven worden als een extra miltje dat ligt op een andere plaats dan waar de milt normaal ligt. De vraag was echter of deze bijmiltjes dezelfde weefsel samenstelling hebben als dat van normaal miltweefsel en of deze ook een vergelijkbare functie konden vervullen.
Van 10 patiënten met normale milten werden bijmilten bestudeerd en vergeleken met de eigenlijke milt. De totale weefselstructuur en de specifieke afweerweefsels en cellen waren identiek aan die van normale milten. Dit suggereert dat de capaciteit om de pneumococcenbacterie te binden en ook een afweerreactie te ontwikkelen hetzelfde kan zijn. Dit zou kunnen betekenen dat bijmilten in staat geacht moeten worden om de immunologische functie van de milt na een miltextirpatie over te nemen, inclusief de mogelijkheid om antistoffen te maken tegen gekapselde bacteriën. Dit heeft dus als consequentie dat bij een operatie deze bijmilten niet moeten worden verwijderd, tenzij met een zuiver therapeutisch doel. Het sparen van de bijmilten kan van betekenis zijn voor de afweer tegen infecties en bloedvergiftiging (sepsis): indien ooit miltverwijdering moet plaatsvinden, b.v. na een ongeval, kan zo'n bijmilt fungeren als "reserve milt".

Hoofdstuk 7. Conclusies

De volgende conclusies kunnen uit het proefschrift getrokken worden:

1 Een ernstig beschadigde milt kan vaak gespaard worden door deze te omwikkelen met een resorbeerbaar net. Dit moet echter beschouwd worden als een specialistische operatie die ervaring vereist.

2 Milt autotransplantaten hebben wel enige activiteit van het filterfunctie systeem (mononucleaire fagocytose), maar te weinig voor een adequate filterfunctie.

3 De milt heeft een belangrijke en unieke rol in het initiëren van de specifieke antistofvormende immuun reactie, met name tegen de zwezerik (thymus) onafhankelijke type 2 antigenen zoals aanwezig in het kapsel van pneumococcen.

4 Autotransplantaten van miltweefsel zijn in staat om een significante verbetering te geven van de specifieke anti-pneumococcen-antistof-vorming (humorale immuun functie) na miltextirpatie.

5 Miltautotransplantaten hebben een normale structuur met normale lymfoïde compartimenten (gebieden met cellen betrokken bij de specifieke afweer), inclusief een functioneel intacte marginale zone.

6 Van autotransplantatie van miltweefsel na miltextirpatie, met name in combinatie met vaccinatie tegen b.v. pneumococcen, kan verwacht worden dat deze herstel geven van de bescherming tegen een vorm van bloedvergiftiging, zoals na een miltextirpatie kan optreden (OPSI syndroom).

7 De functionele anatomische compartimenten van bijmilten verschillen niet van normale milten en hiervan kan worden verwacht dat ze een normale immunologi-

sche functie hebben zoals de milt. Het is te adviseren om bij een routine-operatie, zonder miltpathologie, de bijmilten nooit te verwijderen.

Perspectieven

Aangezien miltletsels niet vaak voorkomen lijkt het verstandig dat deze patiënten opgenomen worden in ziekenhuizen met kennis en ervaring op het gebied van miltsparende behandelingen, zoals gebruik van het resorbeerbare net. In eerste instantie moet gestreefd worden naar het behoud van de milt. Gepoogd kan worden om dit niet-operatief te doen wanneer de patiënt verder in goede toestand is. Indien er wel geopereerd moet worden, is het omwikkelen van de milt met een resorbeerbaar net de eerste keuze. Wanneer een miltextirpatie noodzakelijk is, is het zinvol een autologe transplantatie van miltweefsel uit te voeren. Hierbij moet dan ongeveer de helft van een milt in kleine blokjes gesneden in het grote vetschort voor de darmen (omentum majus) geplaatst worden. Een pneumococcen vaccinatie is dringend aanbevolen, het liefst 3-6 maanden na de operatie en in ieder geval niet eerder.
Als mogelijkheid om de functionele milt-immuun-respons-capaciteit te testen kan een set van miltafhankelijke antigenen gebruikt worden, zoals verschillende typen gezuiverde pneumococcen-kapselbestanddelen. Indien blijkt dat een patiënt specifiek op een aantal subtypen niet reageert kan daar rekening mee worden gehouden bij het optreden van de eerste tekenen van infectie door toedienen van specifieke antistoffen. Ook kan overwogen worden om zulke patiënten specifieke eiwit-geconjugeerde vaccins te geven. Deze vaccins kunnen de aanmaak van antistoffen aanzetten zonder de aanwezigheid van de milt.

Ondanks dat de rol van de milt in het afweersysteem de laatste jaren wel werd erkend, was het niet duidelijk of sparen van de milt bij een ongeval een bijdrage zou leveren ten aanzien van de kans op ziek worden en de kans op overlijden. De resultaten van het onderzoek, beschreven in dit proefschrift, ondersteunen het belang van het sparen van de milt. De onderzoeken met autologe transplantaties van miltweefsel tonen aan dat deze procedure de afweer (immuun respons capaciteit) tegen relevante bacteriën significant verbetert. Verder onderzoek zal moeten uitwijzen wat de optimale hoeveelheid miltweefsel voor de autotransplantaten is en wat het beste moment voor vaccinatie is.
Indien een miltextirpatie onvermijdelijk is bij een patiënt met een miltletsel door een ongeval, moet een autologe transplantatie van miltweefsel in combinatie met een pneumococcenvaccinatie overwogen worden als een valide optie om de kans op overlijden t.g.v. de miltverwijdering te verminderen.

Glossary and abbreviations

Accessory spleen:	A congenital extra spleen somewhere in the peritoneal cavity.
Antibody:	An immunoglobulin molecule which interacts only with the antigen that induced its synthesis in lymphoid tissue or with antigen closely related to it. It is a part of the immunological defence system.
Antigen:	Any substance inducing the formation of antibodies and reacting with the by them induced antibodies. Antigens may be soluble substances (toxins and foreign proteins) or particulates (bacteria and tissue cells). It is something inside the body that does not belong there.
AST:	Autotransplanted splenic tissue.
Autotransplantation:	Taking a piece of tissue or an organ from one part of the body of a subject and inserting in it another localisation in the same individual.
B-cells:	See B-lymphocytes
Billroth's cord:	A non-endothelialized reticular meshwork in the red pulp of the spleen consisting of fibrils and interstitial cells with a large population of monocytes and macrophages.
B-lymphocytes:	"Bursa-equivalent" lymphocytes, thymus-independent (migrating to tissues without passing through or being influenced by the thymus) and matures into plasma cells that synthesise humoral antibody.
B/T-ratio:	The ratio between B- and T-lymphocytes.
Cell-mediated immunity:	Specific acquired immunity in which the role of T-lymphocytes is predominant.
Complement:	A complex series of proteins in serum that interact to combine with antigen-antibody complex producing lysis when the antigen is an intact cell. They are also involved in the generation of anaphylatoxin, the inflammatory response, neutralisation of viruses and participate in other biological activities as antibody-mediated immune lysis, phagocytosis, opsonization and anaphylaxis. There are two pathways of complement; the pathway known longer is called: classical, the one discovered later: alternative.

Ectopic:	Located away from normal position.
EST:	Ectopic splenic tissue.
ELISA:	Enzyme-linked immunosorbent assay. Method for detecting antigens or antibodies utilising enzyme-substrate reactions.
Fc-fragment:	One of the two segments (the constant part): not involved in antigen recognition of the immunoglobulin molecule.
Fc-receptor:	Receptor on a variety of cells for Fc-segment of immunoglobulins.
Fc-receptor test:	A test to measure the presence and capacity of Fc-receptors in the spleen. As Fc-receptors mediate phagocytosis this test is an indirect method to determine the presence of phagocyting cells.
Follicular dendritic cells (FDC):	Cells that form a network around and in between the lymphocytes in the GC. The origin is not clear but the appear to differentiate from reticular mesenchymal cells. They are able to present antigen and play a role in formation and differentiation of B-cells.
F-value:	The ratio of two chi-square values in the Fc-receptor test. Normal (with a biexponential curve) it is 0-0.1 and with a monoexponential curve it is higher.
Germinal centre (GC):	The centre of a secondary lymphoid follicle LF with mainly B-lymphocytes; plays a main role in B-cell selection, isotype switching and affinity maturation
Granulocytes:	Polymorphonuclear leukocytes with abundant granules in the cytoplasm. They have a mainly phagocytosis and a (bacterial) killing function.
Helix pomatia (HPH) haemocyanin test:	Test for evaluating the primary general humoral immunological response (T-cell dependent), after vaccination with HPH.
Helper T-lymphocyte:	T-lymphocyte that co-operates with a B-lymphocyte in antibody formation.
Immune response:	Specific response to antigenic stimulation. A first contact gives primary response and a second contact secondary response.

Immunity: Insusceptibility to the invasive or pathogenic effects of foreign micro-organisms or to the toxic effect of antigenic substances. It can be divided in specific and non-specific immunity.

Immunization: Administration of an antigen in order to bring about an immune response.

Immunodeficiency: An ineffective immune response due to an intrinsic abnormality of B- or T-lymphocytes (primary) or to loss or destruction of antibody and/or lymphocytes (secondary).

Immunoglobulin (Ig): A protein endowed with known antibody activity (e.g. IgG, IgA, IgM), functioning as specific antibody and responsible for the humoral aspects of immunity.

Immunology: Branch of biomedical science concerned with the response of the organism to antigenic challenge.

Interendothelial slit: The slits between the endothelial cells of venous sinusoids in the red pulp of the spleen through which blood flows to enter the venous system. Cells and other elements of the blood can be filtrated by these slits.

Kupffer cell: Macrophage of the liver.

Leucocyte: White blood cell. Major classes: granulocytes, lymphocytes and monocytes.

Lymphocytes: A mononuclear leucocyte, chiefly a product of lymphoid tissue that participates in humoral and cell-mediated immunity. Major classes: B- and T-lymphocytes and natural killer cells.

Lymphoid follicle (LF): Globular structures in the spleen in the white pulp, comprising a specialised reticular meshwork consisting preponderantly of B-lymphocytes and their accessory cells. In the first order the LF is a primary LF with small and medium-sized lymphocytes, after contact with an antigen it becomes a secondary LF with a GC and differentiated large B-cells.

Lymphokines: Soluble protein mediators released by sensitised lymphocytes on contact with antigens, play a role in macrophage activation, lymphocyte transformation, and cell-mediated immunity.

Macrophage:	Highly differentiated mononuclear phagocyte that engulfs and destroys particles.
Marginal zone (MZ):	The filtration bed in the outer border of the secondary lymphoid follicles, forming a zone between the white and the red pulp. It is very richly vasculated and has sinuses.
Monocyte:	Cell of the mononuclear phagocyte lineage.
Mononuclear phagocytes:	Widely distributed macrophages, found in different tissues, and antigen-presenting cells (presenting antigen to lymphocytes).
Mononuclear phagocytic system (MPS):	A dense network with monocytes, macrophages pursed to capture any antigen that has slipped through the nets of other trapping mechanisms. Formerly it was called the reticulo-endothelial system (RES).
Myelocyte:	Immature cell of bone marrow, precursor of polymorphonuclear leukocytes.
Natural killer cell:	Lymphoid cell capable of killing a variety of nucleated cells without antigen stimulation.
Nitroblue tetrazolium test (NBT):	Test for evaluating the phagocyte function of granulocytes. Nitro-blue tetrazolium will be reduced to blue formazan by active granulocytes.
Non-specific immunity:	Immune response that do not involve antigenic stimulation of antibody formation or cell-mediated immunity; it includes phagocytosis, inflammatory response, lysozyme and interferon activity and chemical and physical barriers to infection.
NST:	Non splenic tissue.
OPSI:	Overwhelming postsplenectomy infection.
Opsonization:	Binding of antibodies or complement on the surface of particles to increase their susceptibility to phagocytosis.

Periarteriolar lymphocyte sheath (PALS):	The part of the filtration bed in the g the phagocyte function of granulocytes. Bacterial populations in microtitre trays will be phagocytosed by active granulocytes and the total number of bacterial colonies will be reduced.
Plasma cell:	Differentiated B-lymphocyte that synthesises immunoglobulin.
Polymorphonuclear leucocyte:	White bloodcells having a deeply lobed (3-5) nucleus a way that it appears to be multiple and having cytoplasm containing fine inconspicuous granules. These cells are also called granulocytes. They are capable to migrate outside the bloodvessels and kill and phagocytose antigens and dead cells.
Primary immune response:	The specific antibody response after a first contact with an antigen.
Properdin:	Protein of the alternative complement pathway; binds to C3 convertise and stabilizes it.
Red Pulp:	Spleen tissue consisting of reticular meshwork with sinusoids which is related to the MPS. Performs filter function with large blood flow through this compartment
Reticuloendothelial system (RES):	See: mononuclear phagocytic system.
Reticulum:	A meshwork of reticular cells and reticular fibres, in the spleen constituting filtration beds.
Secondary immune response:	The specific antibody response after a second contact with an antigen. This response will be faster and with a higher antibody production than in the primary immune response.
Specific immunity:	Immune response against a particular disease or a particular antigen (e.g. pneumococ).
Splenosis:	Multiple spontaneous implants of splenic tissue throughout the peritoneal cavity (after a trauma).
T-Cells:	See T-lymphocytes.

T-cytotoxic cells: Subsets of T-cells, when activated, acquire the capacity to lyse target cells carrying antigens. They may also secrete certain lymphokines.

T-helper cells: Are a subset of T-cells which secrete a number of hormonelike proteins (lymphokines) to control and co-ordinate other cells participating in the immune response.

T-lymphocytes: Thymus-dependent lymphocytes (see also under B-lymphocytes) which can suppress or assist the stimulation of antibody production in B-cells in the presence of antigen and can kill such cells as tumour and transplant tissue cells. They are responsible for cell-mediated immunity and immunological memory. See also under a T-helper, T-cytotoxic and T-suppressor cells.

T-suppressor cells: T-cells secreting, upon activation, molecules that inhibit the response of other cells.

Tuftsin: A tetrapeptide originating from the Fc-fragments of IgG which stimulates the phagocyte activity of polymorphonuclear leucocytes (granulocytes).

White pulp: Reticular meshwork with selective filters, lymphocytes and accessory cells, setting them up to carry out specific immune responses. It consists of PALS, LF and MZ.

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CURRICULUM VITAE

1966 Nautical college Willem Barentsz, West Terschelling, navigating officer (BS) merchant marine

1970 Shipping-company Royal Nedlloyd, Rotterdam

1972 Certificate third rank

1971 Navigating officer Royal Navy Reserve, Den Helder
Chief-officer minesweepers

1974 Medical study, State University Groningen

1981 Medical degree
Resident internal medicine and surgery, Bonifatius Hospital Leeuwarden

1982 Resident radiotherapy, University Hospital Groningen

1983 Resident surgery, University Hospital Groningen

1988 Certificate general surgery
Post-graduate course traumatology, University Hospital Groningen

1990 Consultant traumatology and vascular surgery, Medical Centre Leeuwarden,
Location North

1998 Consultant traumatology and vascular surgery and medical co-ordinator emergency department, Medical Centre Leeuwarden, location South.

Address for correspondence

R. Leemans
Department of surgery
Medical Centre Leeuwarden
Henri Dunantweg 2
8943 AD Leeuwarden
The Netherlands
Phone : +31 (0)58 286 6162
Fax : +31 (0)58 286 6946
E-mail : rleemans@knmg.nl

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